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THE UNIVERSITY OF ALBERTA
A VOLTAGE CLAMP SYSTEM FOR MYELINATED NERVE FIBRES

by



ROBERT WALTER HUCK

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
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DEPARTMENT OF ELECTRICAL ENGINEERING

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UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "A Voltage Clamp System for Myelinated Nerve Fibres", submitted by Robert Walter Huck in partial fulfilment of the requirements for the degree of Master of Science.

THE HISTORY OF THE
CITY OF BOSTON

The history of the city of Boston is a subject of great interest to all who are connected with it. It is a city of many centuries, and its history is a record of the progress of civilization. The city was founded in 1630, and has since that time been a center of commerce and industry. It has been the seat of many important events, and has played a prominent part in the history of the United States. The city is now one of the most important cities in the world, and its history is a record of the progress of the human race.

ABSTRACT

Voltage clamp experiments have become very useful in examining the mechanisms by which ionic fluxes cross the excitable membranes of nerve fibres. This thesis examines the potentiometric technique of voltage clamping myelinated nerve fibres. In this technique electronic negative feedback is applied across petroleum jelly seals strategically placed along the nerve axon.

The necessary electronic control and measurement circuits were designed and constructed. Also the necessary apparatus for mounting the nerve fibres for experimental purposes was constructed.

The total system was examined analytically to investigate sources of error and to study the stability of the voltage control circuits.

The system so designed and analyzed appears to be suitable and quite reliable for experimentation with myelinated nerve fibres.

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CHAPTER I

INTRODUCTION

Understanding of excitable nerve fibres has improved tremendously due to the development of the voltage clamp technique. This method provides a means of controlling some of the electrical characteristics of the membranes and, as a result, allows one to more easily carry out detailed studies on the ion fluxes across active membranes.

It is perhaps appropriate that voltage clamping be formally defined. The definition involves an ideal voltage clamp situation and, as such, serves as a basis for the evaluation of the various techniques. In this manner, the various problems encountered in the non-ideal experimental environment can then be more readily appreciated.

A definition of voltage clamping can be stated as follows: A membrane is under a voltage clamp condition when the potential across the electrically excitable membrane has at all times a known and controlled value. Ideally this potential difference is constant over the area of membrane through which current flow is measured.

In the actual implementation of a voltage clamp on a membrane, lack of perfect electrodes and the need to avoid damage to the membrane, prevents one from ever achieving ideal experimental conditions. Consequently, it is necessary to be constantly critical of the experimental conditions, requirements and the results obtained with any voltage clamp technique.

Voltage clamp techniques have been developed for various sizes of both myelinated and large non-myelinated fibres. The following is a list of these techniques:

1. Electrodes inserted longitudinally into large non-myelinated axons.¹
2. Micropipette electrodes inserted transversely into the axon.²
3. Sucrose-gap technique.^{3, 3a}
4. Air-gap technique.⁴
5. Potentiometric system using negative feedback around petroleum jelly seals.^{5, 6, 7}

The first three techniques listed above were developed primarily for large non-myelinated fibres. The sucrose-gap technique, however, could be used for much smaller non-myelinated fibres than the other two. The last two techniques were developed for use on myelinated nerve fibres. The potentiometric system was by far the most reliable and for this reason a study of this technique has been made.

Before proceeding with the system design and analysis it is appropriate that a detailed explanation of this technique be made.

This technique was first employed by Frankenhaeuser⁵ to measure action potentials and various other electrical properties of small 15-20 micron diameter myelinated nerve fibres. It was then extended to voltage clamping experiments by Dodge and Frankenhaeuser,⁶ Moore,⁷ and a few others.

Voltage clamp by this procedure relies on the ability to prevent longitudinal current flow down the axon by means

of electronic negative feedback. The negative feedback artificially increases the resistance of petroleum jelly seals formed around the internodes. Figure 1.1 illustrates the experimental arrangement with the necessary electronics to achieve voltage clamping.

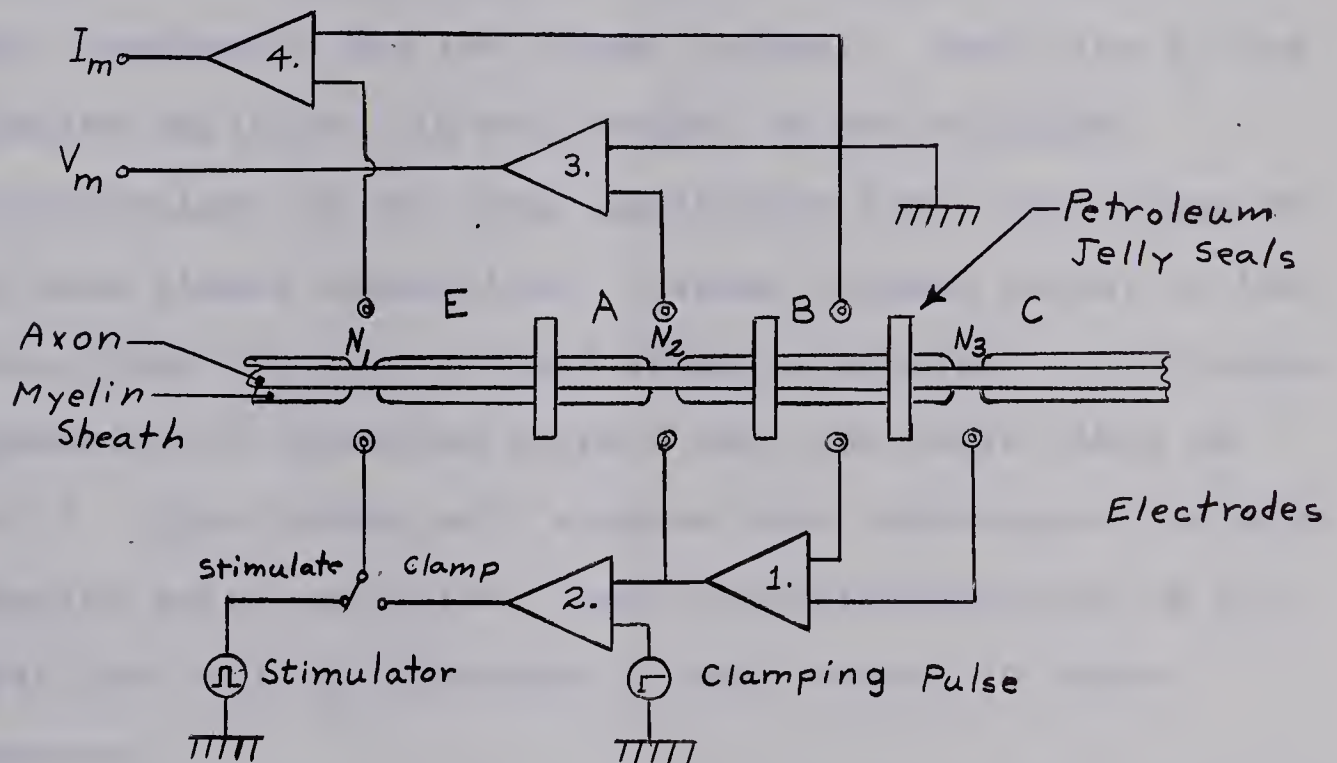


Figure 1.1

Voltage Clamp System with Electronic Negative Feedback Around External Seals

The nerve fibre is mounted in a specially constructed chamber such that petroleum jelly can be used to form seals around the fibre resulting in pools E, A, B and C. Pool E is the pool into which current is injected to stimulate or clamp the active node and contains a depolarized node, N_1 . Pool A contains the node under investigation, N_2 . Pool B contains a portion of the internode between nodes N_2 and N_3 and is at ground potential with respect to the amplifier system. The last pool, pool C, contains another node, N_3 ,

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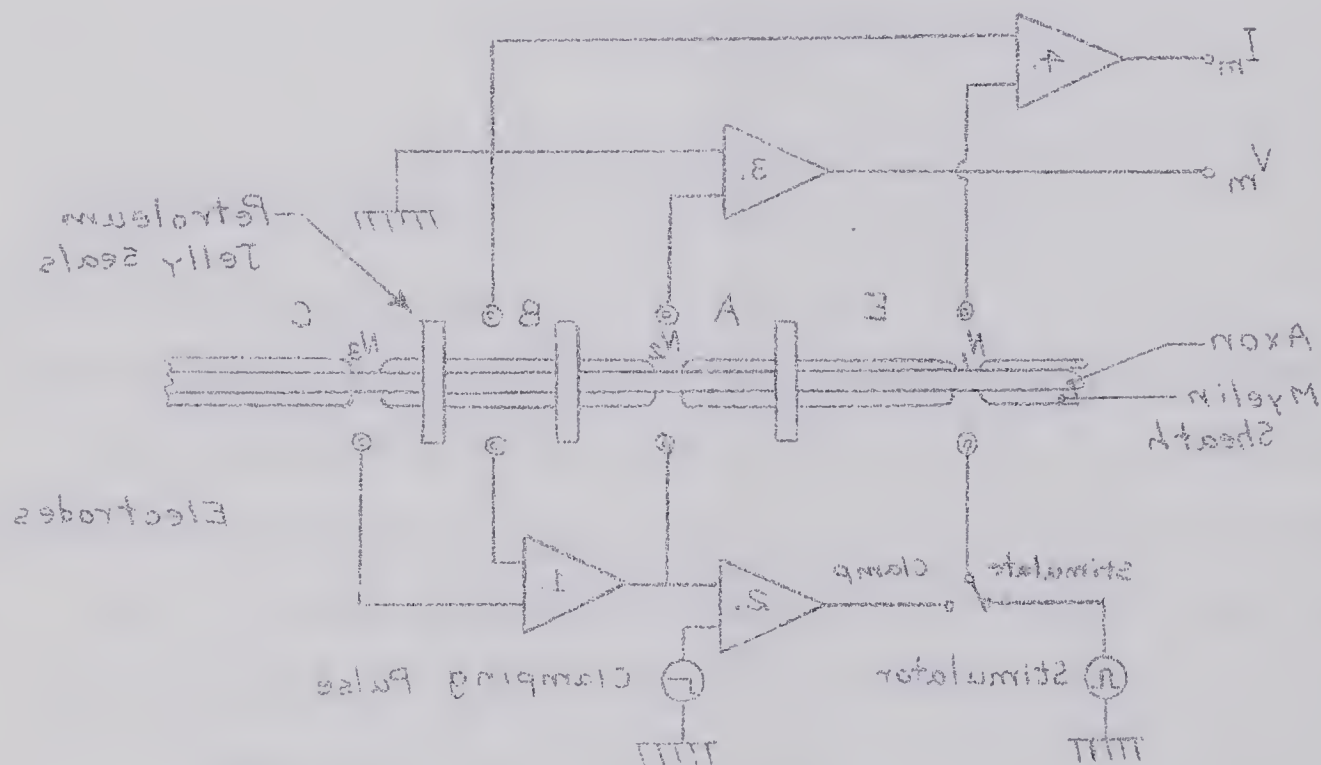


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which is depolarized, as is N_1 , by isotonic KCl.

Electrolytic connections are made between the pools and calomel half cells by means of salt bridges constructed from glass capillary tubes filled with saturated KCl - agar gel. The amplifiers are then connected directly to the calomel cells. Amplifiers 1, 3 and 4 require very high input impedances and low offset current. Amplifier 2, the clamping amplifier, is not subject to the stringent specifications of the other amplifiers since the inputs do not make direct connections, through calomel cells, to the nerve fibre itself. It does require, however, a low output impedance for injecting current into the nerve fibre in pool E. This thesis will examine this technique of voltage clamping quite extensively and the characteristics of the amplifiers will be discussed in more detail in later chapters.

Frankenhaeuser⁵ first used this technique to measure resting potential, nodal resistance and capacitance and action potentials. The electronic system used for this was essentially as shown in Figure 1.1 except Amplifier 2 was not included. Two characteristics of the system which should be noted are: (1) The output signal is to a large extent independent of the gain in the feedback amplifier (provided that the gain is large); (2) The output signal approaches the input signal in amplitude and time course. To analyze the potentials developed in the system it is necessary to define some parameters.

V_{BC} = Voltage across B-C seal impedance, Z_{BC}

V_{AB} = Voltage across A-B seal impedance, Z_{AB}

D is a point inside the fibre at the active node, N_2

V_{CD} = Voltage across Z_{CD} , the internal longitudinal impedance between nodes N_2 and N_3 , plus the transverse impedance at node N_3

V_{AC} = Voltage between points A and C across Z_{in} the amplifier system input impedance.

G = Voltage gain of Amplifier 1

The following equations can be written:

$$V_{BC} = V_{AC} - V_{AB} \quad (1.1)$$

$$V_{AB} = G \times V_{BC} \quad (1.2)$$

$$\text{Hence } V_{AB} = \frac{G \times V_{AC}}{G + 1} \quad (1.3)$$

The potential at the input of the amplifier is then

$$V_{BC} = V_{AC} - \frac{G \times V_{AC}}{G + 1} \quad (1.4)$$

The current relationship in the system is

$$\frac{V_{BC}}{Z_{BC}} = \frac{V_{AC}}{Z_{in}} \quad (1.5)$$

From equations 1.4 and 1.5 the amplifier system input impedance can be obtained as

$$Z_{in} = Z_{BC} (G + 1). \quad (1.6)$$

Considering the series impedance Z_{CD} , it follows that

$$V_{AC} = V_{in} \frac{Z_{in}}{Z_{in} + Z_{CD}} \quad (1.7)$$

where V_{in} is the input signal.

Substituting equation 1.7 into equation 1.3 gives

$$V_{AB} = V_{in} \frac{G z_{BC}}{z_{CD} + (G + 1) z_{BC}} . \quad (1.8)$$

Using typical values of $G = 1000$, $z_{CD} = 50 \text{ M}\Omega$, and $z_{BC} = 10 \text{ M}\Omega$ it follows that V_{AB} differs from V_{in} by less than 0.5 per cent.

From the above analysis it is apparent that one can measure the transmembrane potential in the nodal region by monitoring the voltage across the seal between pools A and B. By injecting a short burst of current into the active node through the node N_1 , depolarized by KCl, an action potential can be measured across seal A-B. By injecting a prolonged subthreshold current into node N_2 one can determine to a fair degree of accuracy the membrane time constant. Finally, membrane resting potential can be measured by first balancing the amplifier with the entire nerve fibre bathed in Ringer's solution. Then, by replacing the Ringer's solution in pool A by isotonic KCl, the resting potential can be measured across seal A-B. As in the other cases the whole of the axis cylinder is kept by the feedback system at a constant potential and the change in transmembrane potential appears as a change in the voltage across seal A-B.

The voltage clamping capabilities of the system were added later by Dodge and Frankenhaeuser⁶ and, most recently, Moore⁷ merely by incorporating another D.C. differential

input amplifier, Amplifier 2, into the existing system. One input of this clamping amplifier was connected to the transmembrane voltage measuring system previously described while the other was connected to the clamping voltage generator. The output of this amplifier produced a current through node N_2 such that the transmembrane potential was equal in magnitude and opposite in phase to the clamping voltage.

Amplifier 1 prevented any appreciable current flow between nodes N_2 and N_3 while Amplifier 2 produced a sufficient current flow between nodes N_1 and N_2 to stabilize the transmembrane potential. Consequently, almost all the current associated with step depolarizations flowed entirely through the internode between nodes N_1 and N_2 . A measure of this current which is very close in value to the current flowing through node N_2 could be obtained by measuring V_{ED} and calculating current density from

$$I_m = \frac{V_{ED}}{A_N z_{ED}} \quad (1.9)$$

where A_N = the surface area of node N_2

z_{ED} = the impedance between pool E and the point D inside the fibre.

V_{ED} , however, cannot be measured directly. Since the point D is held constant relative to ground, and in fact very close to ground, any changes in transmembrane potential appear as changes in potential between pool A and the grounded pool B. Because of this and because the potential

between pools A and B is held constant by the voltage clamp, the current variations flowing through the node would then appear as voltage variations between pools E and B. Since V_{ED} is almost equal to V_{EB} , recording V_{EB} and dividing by an estimated value for $A_N \times Z_{ED}$ will indicate the value of current density flowing through the membrane at node N_2 .

With the total system operating as described, the functioning of the node can be determined under various conditions of temperature, ionic concentrations of solutions, ionic content of solutions, etc. This technique has been employed most successfully of any technique to date to examine myelinated nerve fibres.

There are four areas in which limitations imposed on the system were of most importance in experiments carried out to date. They include the speed of stabilization, attenuation in the voltage recording system, oscillations, and deterioration in the nerve fibre. Some concern was placed on the polarization and drift of electrode and amplifiers; however, this effect was minimized by proper design, or in some cases selection, of the amplifiers and the use of calomel cell electrodes.

The speed of stabilization depended somewhat on the width of the seals and the pools in the recording cell. Various tests were conducted on recording cells with different size pools. After some experimentation it was concluded that the speed of stabilization was most rapid when a recording cell with short seals and pools was used.

A problem in the attenuation of the voltage recording system appeared to depend on the size of the pools in the recording cells. When a short length of fibre was in pool B the transmembrane potential was attenuated by as much as 30 per cent. This was decreased to a negligible amount when the pool was widened by 100 microns. Consequently, in choosing the size of the recording cell, a compromise had to be made between the requirements of the speed of stabilization and the requirements of the potential measuring system.

Another difficulty arising in this technique occurred due to oscillations in the electronic control system. The possibility of oscillations occurring in the system increased with the speed of stabilization. The majority of phase lag in the system occurred in the nerve fibre between pools E and C. In order to minimize the total phase lag, the recording cell could be made small; however, once again the size could not be decreased to the point where there was any appreciable attenuation in the recording system.

One last consideration is concerned with the deterioration of the nerve fibre during the course of an experiment lasting a few hours. The effect observed was a decrease of the maximum inward current and a shift of the potential at which the initial current reversed from inward to outward. Fibre deterioration was attributed to the larger current flow under clamped conditions than in normal pulse activity and to the tight packing of petroleum jelly around the fibres.

CHAPTER II

OBJECTIVES OF THESIS

In the past the majority of the more detailed investigations carried out on nerve fibres have been done on non-myelinated fibres. Relatively easily implemented internal electrode systems used on the non-myelinated fibres allow one to study these fibres much more readily than those of the external electrode systems. Because of the more detailed investigations carried out on the non-myelinated fibres, the measuring system has been well analyzed to determine sources of error and to determine also the range of applicability. This thesis attempts to analyze the last of the clamping techniques described in the previous chapter; that of electronic negative feedback across external petroleum jelly seals. As described previously, this technique of applying external electrodes was designed for use on the much smaller myelinated nerve fibres.

In order to carry out this study, a voltage clamping system employing this technique had to be constructed. Accuracy specifications were determined for the measuring and control amplifiers and the system was designed on the basis of these specifications. The system required four D.C. differential operational amplifiers, three with high input impedance, low offset voltage and current, and low temperature drift. Also required were a voltage generator for supplying both stimulation and clamping pulses, and an

electrode and tank assembly to allow connection to the various regions of the nerve fibres.

The second portion of the thesis involves a detailed analytical study of the system's operating characteristics. The system was examined mathematically to determine possible sources of error and possible limitations affecting the measurements due to the method of implementing the transmembrane potential control and the voltage clamping control. A large portion of the analytical study concerned the determination of the stability characteristics of both the potential control circuit and the total clamping system. This study involved extensive use of the University of Alberta's IBM 360/67 computer system using the APL language.

One further consideration which was examined analytically was the attenuation in the action potential measuring system due to a short length of internode in the grounded pool. The effect of this attenuation on the stability of the potential control system was examined.

Experiments were carried out in an effort to obtain data to confirm the results of the calculations. This proved to be a difficult task as it is with almost any physiological experiment. Limited data was obtained, however, which could be applied.

The intention of this thesis was to complete the above tasks to allow a better insight into the accuracy and reliability of data obtained from myelinated nerves and to allow a better understanding of the system performance

so that one can evaluate the system's applicability in various experimental conditions.

CHAPTER III

EQUIPMENT DEVELOPMENT AND SYSTEM ANALYSIS

A. Equipment Design

1. Specifications

(a) Potential Control Amplifier

The transmembrane potential control and measurement system amplifiers are the most crucial of those employed in the overall system. The measurement system is made up of two amplifiers, one to supply the negative feedback to the active node in pool A to effectively prevent any appreciable current flow along the internode, and the other to measure the potential between pools A and B. Of these two amplifiers, the potential control amplifier (Amplifier 1 of Figure 1.1) is the most important.

The specifications necessary for this amplifier were determined from an accuracy requirement arbitrarily selected and from the impedances of the nerve fibre and external seals. The accuracy was selected to be within ± 1 mv of the true transmembrane potential. Due to the variations in physical size and consequently the variations in impedances of the nerve fibre, an accuracy greater than this would not be required.

Consider the nerve fibre with its D.C. equivalent circuit illustrated in Figure 3.1. V_m is the transmembrane potential at the node, R_{ax} is the axial

resistance along the internode and R_s is the seal resistance. From previous experiments approximate

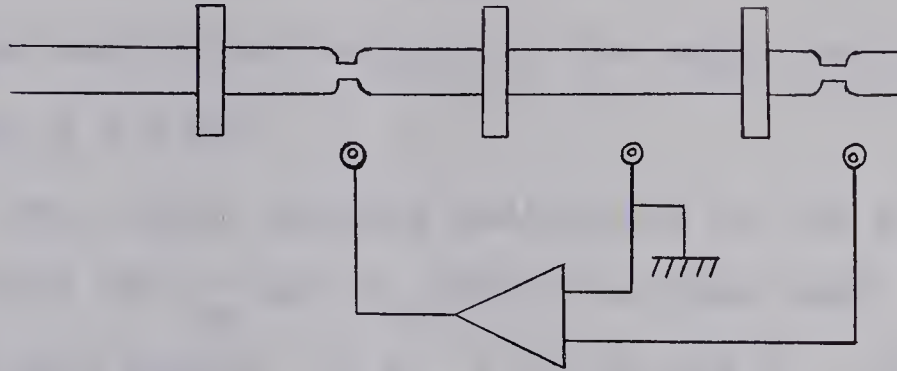


Figure 3.1(a)

Nerve Fibre with Seals
and Potential Control Amplifier

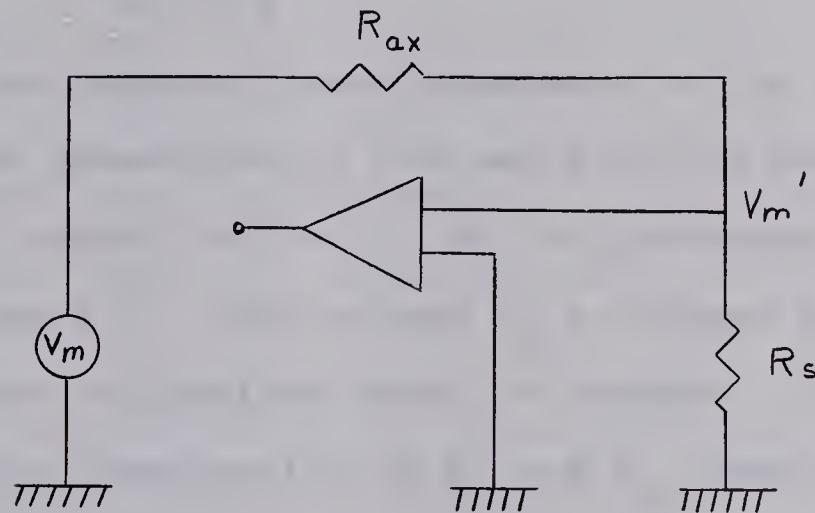


Figure 3.1(b)

D.C. Equivalent Circuit

values of R_{ax} and R_s are 20-50 M Ω and 5-10 M Ω respectively. The values that these resistors were assigned in the calculation of each of the specifications were selected from within these ranges to give the 'worst case' combination. To determine the allowable input offset voltage the values $R_{ax} = 50$ M Ω and $R_s = 5$ M Ω were used.

From Figure 3.1(b)

$$\Delta V_m' = \frac{5}{5 + 50} \Delta V_m \approx \pm 0.1 \text{ mv} \quad (3.1)$$

Therefore the offset voltage of the amplifier should be less than $\pm 0.1 \text{ mv}$.

The offset current multiplied by the parallel combination of R_{ax} and R_s should be less than $\pm 0.1 \text{ mv}$ for the same reason. If $R_{ax} = 50 \text{ M}\Omega$ and $R_s = 10 \text{ M}\Omega$, this will then require that the offset current, I_0 , be

$$I_0 = \frac{0.1 \text{ mv}}{\frac{R_{ax} \times R_s}{R_{ax} + R_s}} \approx \frac{0.1 \text{ mv}}{10 \text{ M}\Omega} \approx 10^{-11} \text{ Amps} \quad (3.2)$$

The required input impedance to the amplifier system was determined on the basis of the maximum allowable error, $\Delta V_m' = 0.1 \text{ mv}$, as previously calculated by equation 3.1. This allows R_s to change by 10 per cent before the maximum error is reached. Consequently, the parallel combination of R_s and R_{in} should be greater than $0.9 R_s$. Hence,

$$\frac{R_{in} \times R_s}{R_{in} + R_s} = 0.9 R_s \quad (3.3)$$

Solving the equation for R_{in} , with $R_s = 10 \text{ M}\Omega$, R_{in} is required to be greater than $100 \text{ M}\Omega$.

The gain of the amplifier was selected to be variable from 0 to 1000. An adjustable gain was required

such that the gain could be set to zero while the system was being connected to the nerve fibre. This prevented transients from being amplified, which would have resulted in the destruction of the fibre. The requirement for the maximum gain of 1000 was discussed on page 6.

The frequency bandwidth for the system was selected to be from D.C. to 2000 Hz. This bandwidth will be shown later to be adequate.

The equivalent input offset voltage drift was selected to be $\leq 10 \mu\text{V} / ^\circ\text{C}$. This allowed the temperature to vary $\pm 10^\circ\text{C}$ before the maximum error of 0.1 mV at the amplifier input was reached. These experiments must all be done under laboratory conditions and this temperature range was consequently more than adequate.

This amplifier also required an adjustable D.C. level of -0.5 volts to + 0.5 volts. This was necessary to permit the examination of the fibre's ionic activity under varying D.C. biasing conditions.

These specifications can be summarized as follows:

1. Input Offset Voltage $\leq 0.1 \text{ mV}$
2. Input Offset Current $\leq 10^{-11} \text{ Amps.}$
3. Input Impedance $\geq 100 \text{ M}\Omega$
4. Gain $0 \rightarrow 1000$
5. Frequency Bandwidth : D.C. - 2000 Hz

6. Equivalent Input Offset

Voltage Drift $10 \mu\text{V} / \text{C}^\circ$

7. Adjustable D.C. level: -0.5 volts to
 $+ 0.5$ volts

(b) Potential Measuring Amplifier

Since this amplifier (Amplifier 3 of Figure 1.1) is part of the potential measuring system and since it is also measuring the potential difference across a high impedance seal, it requires specifications identical to those of the previous amplifier except for the following:

1. A gain of 10 was chosen for this amplifier to give a convenient scale factor for displaying the potential variations on the oscilloscope. The potentials being measured were in the order of 100 mv. Thus a gain of 10 permitted recording of signals in the 1 volt range.
2. The frequency bandwidth was selected to be from 0 to $\geq 10\text{KHz}$. This provided a more than adequate bandwidth for recording the output signals as will be indicated later by the experimental results.
3. No adjustable D.C. level was required.

For the remainder of the specifications the reader is referred to those of the potential control amplifier.

(c) Clamping Amplifier

This amplifier (Amplifier 2 of Figure 1.1), used to clamp the transmembrane potentials to values as determined by the voltage generator system, is a differential input D.C. operational amplifier. It does not require the stringent input specifications as do the others because both inputs are connected directly to the low impedance outputs of other operational amplifiers. Motorola's MC1439G operational amplifiers were more than adequate to serve this purpose. Since both inputs to the clamping amplifier were connected to low output impedance operational amplifiers, the input impedance did not have to be very high. Also considered in selecting the input impedance was the requirement for adjusting the gain from 0 to 100. A 1000 Ω input resistance was sufficient allowing the variable feedback resistor to be 100 K Ω .

The input offset voltage was required to be ≤ 1 mv in order to maintain the transmembrane voltage within the required accuracy of ± 1 mv.

The input offset current was not a critical consideration. A current of $I_0 = \frac{1 \text{ mv}}{60 \Omega} \approx 1.5 \times 10^{-5}$ Amps could be tolerated before the maximum error was reached.

The closed loop gain of the amplifier was required to read a maximum of 100. It was necessary to be able to adjust the gain from 0 to 100 for the reasons discussed in potential control amplifier specifications.

The maximum gain of 100 would, of course, provide for a voltage clamp to within 1 mv for a 100 mv clamping pulse.

The frequency bandwidth was selected to be from D.C. to 20KHz. This provided a sufficiently fast response for the system when subjected to a step input. The nerve fibre time constants are quite large as indicated by the experimental results.

One last consideration for this amplifier was the equivalent input offset voltage drift. This was selected to be a maximum of $10 \mu\text{v} / \text{C}^\circ$ for the reasons discussed previously.

The required specifications can be summarized as follows:

1. Input Impedance $\geq 1000 \Omega$
2. Input Offset Voltage $\leq 1 \text{ mv}$
3. Input Offset Current $\leq 1.5 \times 10^{-5} \text{ Amps}$
4. Gain $0 \rightarrow 100$
5. Frequency Bandwidth D.C. to $\geq 20 \text{ KHz}$
6. Equivalent Input Offset
Voltage Drift $\leq 10 \mu\text{v} / \text{C}^\circ$

(d) Current Measuring Amplifier

The current measuring amplifier (Amplifier 4 of Figure 1.1) required specifications identical to those of the voltage measuring amplifier with one exception. The gain required to serve as a suitable scale factor

for oscilloscope display was increased to 100. As indicated on page 7 the current is determined from the voltage across Z_{ED} . Since the current is in the order of $10 \text{ ma} / \text{cm}^2$ and Z_{ED} is approximately $10 \Omega \text{ cm}^2$ the resulting voltage is in the order of 100 mv. Amplification by 100 increased this voltage to a size more easily displayed on the oscilloscope. For the remainder of the specifications the reader is asked to refer to those of the voltage measuring amplifier.

(e) Voltage Generator System

This system was designed and constructed to provide the stimulating and clamping pulses used on the nerve fibres. The specifications and necessary provisions for this system are listed below:

1. Risetime $\leq 10 \mu\text{sec}$. This provided a step input sufficiently faster than the transient response of the nerve fibre.
2. Frequency Range : 0.1 Hz - 5 KHz. A frequency range of this amount provided for the frequencies of physiological interest.
3. Pulse Width Range :
 Stimulating Pulse - 50 μsec to 4 msec
 Clamping Pulse - 4 msec to 400 msec
 These pulse widths were again selected because they were in the area of physiological interest.

4. Output Impedance $\approx 60 \Omega$. Since the voltage generator system was required to occasionally inject large amounts of current this small output impedance was required.
5. Provision to switch between stimulating and clamping mode.
6. Provision to switch between a positive and negative output.
7. Provision for automatically sequencing pulse amplitude in uniform voltage increments of 5, 10, 20 or 40 mv.
8. Provisions to start and stop the automatic sequencing and the entire system at any increment.
9. Provision to switch between the automatic pulse amplitude sequencing system and a manual amplitude control.
10. Provision to produce a single pulse.

(f) Nerve Mounting Tank

The clamping technique requires that the nerve mounting tank be constructed from Lucite to the dimensions as indicated in Figure 3.2.

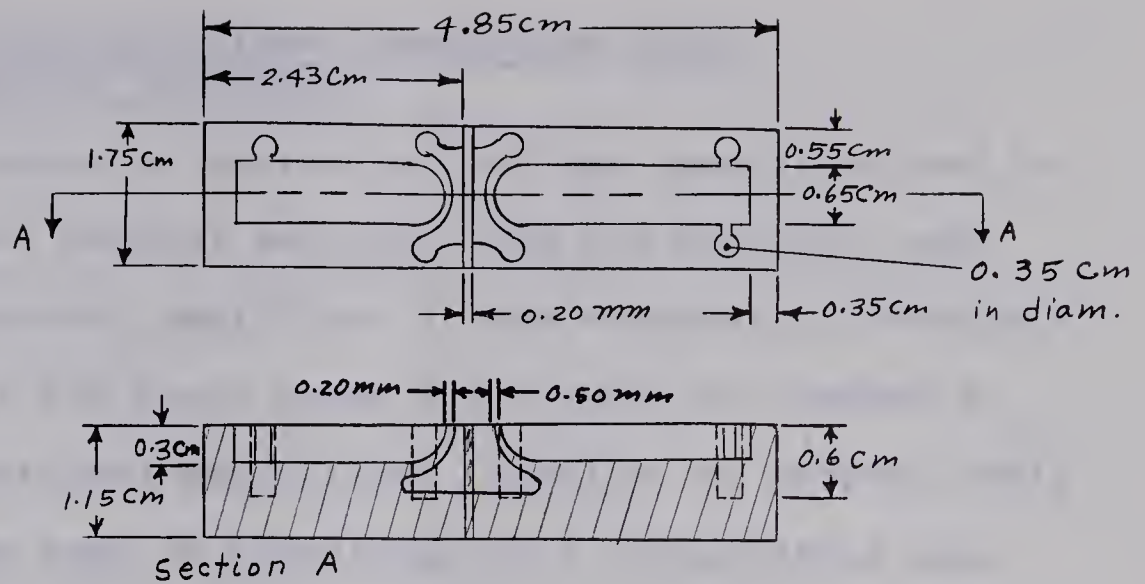


Figure 3.2

Nerve Mounting Tank

In order to connect the four pools as illustrated in Figure 3.2 to the electronic amplifiers, seven calomel cells and salt bridges were required. The impedance of the calomel cell-salt bridge combination was required to be $< 1000 \Omega$ to prevent any appreciable error in the recording amplifiers. This error was then kept below 0.1 per cent.

Shielding was required to minimize capacitive coupling between the calomel cell electrodes. Interference by capacitive coupling could result in error and, if strong enough, oscillations which would destroy the fibre.

2. Design of Field Effect Transistor (FET) Differential Amplifiers

In order to achieve all of the specifications for the potential control amplifier and the potential and current measuring amplifiers it was necessary to design a differential FET input stage which could be adapted to normal operational amplifiers. Ideally, to operate FET's or any other type of transistor in a differential pair configuration, the transistor characteristics should be perfectly matched. In this manner, unwanted effects such as temperature drift in one transistor due to variations in operating environment or current levels would be cancelled by the same effects occurring in the other transistor. This, of course, is one of the major advantages of differential input amplifiers. A complete cancelling effect occurs only if the transistor characteristics are perfectly matched. In practice, an ideal or perfect match can not be achieved, however, it can be approximated very closely.

A study of FET characteristics was made to determine the basis on which two FET's should be matched for differential applications and also to determine the proper procedure for biasing the FET's. The obvious way to match two FET's by selection is to match the transfer characteristics, measured at the same temperature, relating the gate-to-source voltage, V_{GS} , and the drain current, I_{DS} . Determining a number of FET transfer characteristics by

laboratory measurement and matching the characteristic with that of another FET would, however, prove to be a very laborious task.

Equation 3.4 approximates very closely the transfer function of the FET.

$$I_{DS} = I_{DSS} \left(1 - \frac{V_{GS}}{V_P} \right)^2 \quad (3.4)$$

where I_{DS} = drain current

I_{DSS} = zero-gate-voltage drain current

V_{GS} = Gate-source voltage

V_P = Gate-source pinch-off voltage

From equation 3.4 it is evident that the transfer characteristics are defined by the two parameters, I_{DSS} and V_P . By measuring these two values for several FET's under the same temperature conditions and selecting the two FET's in which their values are closest, the desired match of the transfer characteristics is achieved. This match, of course, will depend on how close the parameters match and how accurately they were measured.

One further parameter must be matched before the two selected FET's are suitable for critical applications, particularly if the impedance of the driving source is high. This parameter is I_{GSS} , the gate cut-off current, or more commonly, the gate-source leakage current.

For the FET's used in Amplifiers 1, 3 and 4 of Figure 1.1, I_{DSS} and I_{GSS} were measured directly using the test conditions as indicated in the Texas Instruments

Incorporated specification sheet for the 2N3819 N-channel planar silicon field effect transistor. The pinch-off voltage was defined in equation 3.4 as the gate-source voltage to give $I_D = 0$ and is determined by measuring V_{GS} when $I_D = 0.1 I_{DSS}$.

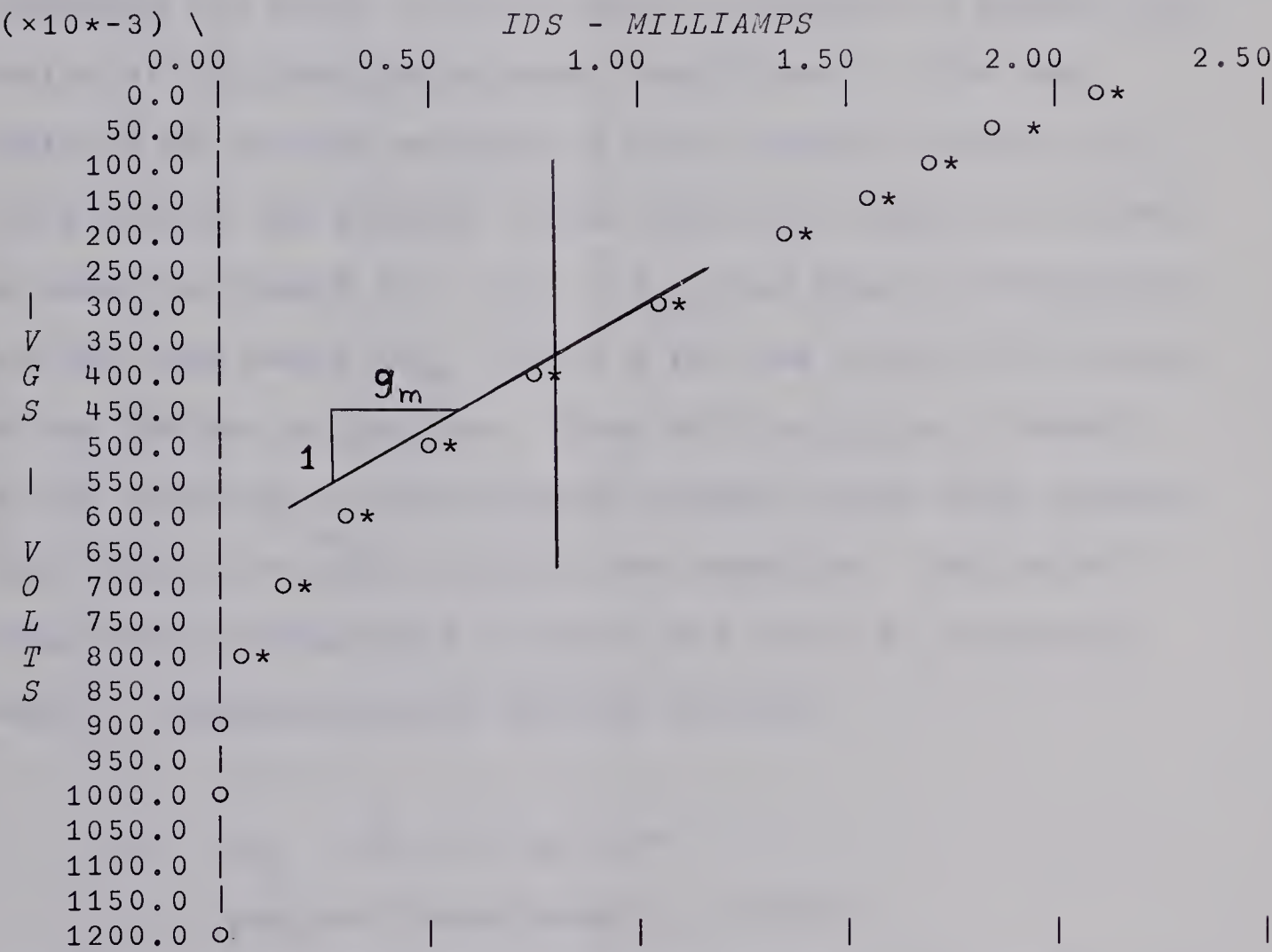
$$V_p = 1.46 V_{GS} \quad \left| \quad I_D = 0.1 I_{DSS} \right. \quad (3.5)$$

In this manner three pairs of FET's were selected. For each pair the actual transfer characteristics were then measured and plotted in order to determine the transconductance, g_m . Since the design procedure is exactly the same for the FET input stages of all three amplifiers, only one design will be discussed to serve as an example for the rest. The plotted transfer characteristics and the determination of the transconductance, g_m , are illustrated in Figure 3.3 for the two FET's selected and used in the potential control amplifier.

The FET characteristics, particularly those associated with temperature variations, were then examined to determine the optimum biasing currents. FET's exhibit a very useful characteristic in that at a certain operating current the temperature coefficient is zero. This is illustrated in equation 3.6 derived by Sevin⁸ by the fact that the right hand side of the equation can go to zero.

$$\frac{\partial V_{GS}}{\partial T} = \frac{dV_p}{dT} - \frac{nV_p}{2T} \sqrt{\frac{I_D}{I_{DSS}}} \quad (3.6)$$

FIELD EFFECT TRANSISTOR TRANSFER CHARACTERISTICS
TEMPERATURE - 295 DEGREES KELVIN



MATCHED PAIR FIELD EFFECT TRANSISTORS
○ FET NO. 1
* FET NO. 2

FIGURE 3.3

Elimination of first order temperature variations can be achieved by taking advantage of this fact and operating the FET's at the zero temperature coefficient point.

The next step in the design procedure was to determine the value of bias current required to operate the device at its zero temperature coefficient. This was achieved by solving equation 3.6 for various values of V_p and plotting the results in the form of a family of curves as shown in Figure 3.4. I_D / I_{DSS} can then be determined for the case where $\partial V_{GS} / \partial T = 0$ for the pinch-off voltage of the device in question. This will only give a result in the vicinity of the required current since some assumptions had to be made to solve the equation. The actual value can be determined by trial and error as explained later. Assumptions were made as follows:

1. $n = 2$
2. $dV_p / dT = -2 \text{ mv} / ^\circ\text{C}$
3. Ambient Temperature $T = 295^\circ\text{K}$

The first two assumptions are the values for n and dV_p / dT which are generally accepted for this type of FET. Applying them to individual transistors may result in some error, however, probably less than that involved in determining V_p .

The values of I_{DSS} , V_p and I_{GSS} for the two FET's used in the design were as follows:

FIELD EFFECT TRANSISTOR TEMPERATURE CHARACTERISTICS
DRIFT REFERRED TO INPUT VERSUS I_D/I_{DSS}
TEMPERATURE - 295 DEGREES KELVIN

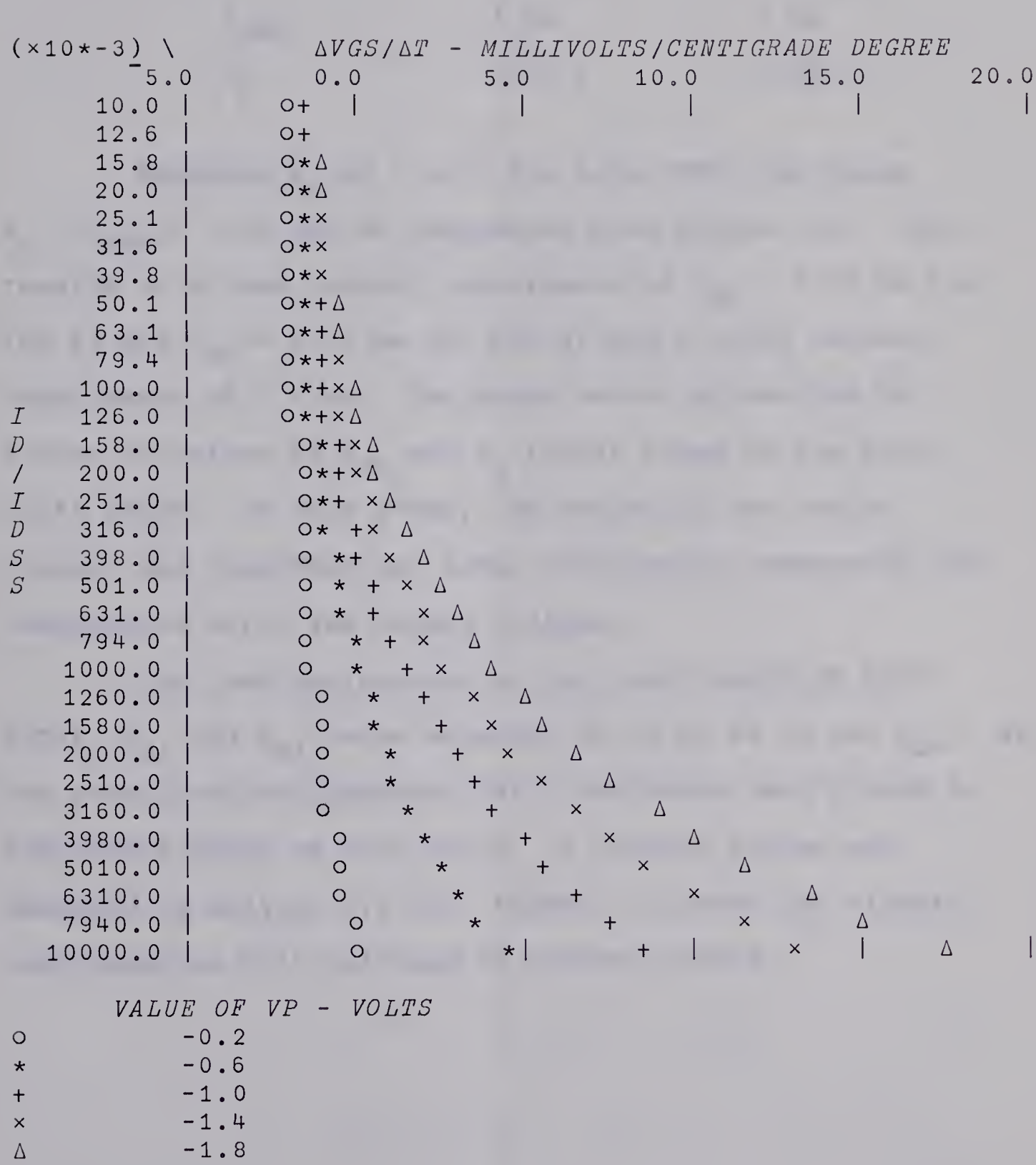


FIGURE 3.4

	<u>FET #1</u>	<u>FET #2</u>
I_{DSS}	2.08 ma	2.13 ma
I_{GSS}	1 pa	1 pa
V_p	0.95 v	0.99 v

Rounding V_p to 1 volt for both FET's the value $I_D / I_{DSS} = 0.38$ can be determined from Figure 3.4. This results in a bias current requirement of $I_{D1} = 0.79$ ma for FET #1 and $I_{D2} = 0.81$ ma for FET #2 and a total current requirement of 1.6 ma. The above method allows one to arrive at values of V_{GS} and I_D fairly close to the zero drift point. At this stage, the design of the entire circuit was completed and later modified to compensate for temperature drift and offset voltage.

The load resistances in the drain leads of both FET's, R_{D1} and R_{D2} , were selected to be $10\text{ K}\Omega$ to set $V_{DS} \approx 4\text{v}$. For stabilization purposes, $220\ \Omega$ resistors were placed in the source leads of both FET's. A current source was designed to deliver 1.6 ma. Figure 3.5 shows the circuit configuration with voltages at various points.

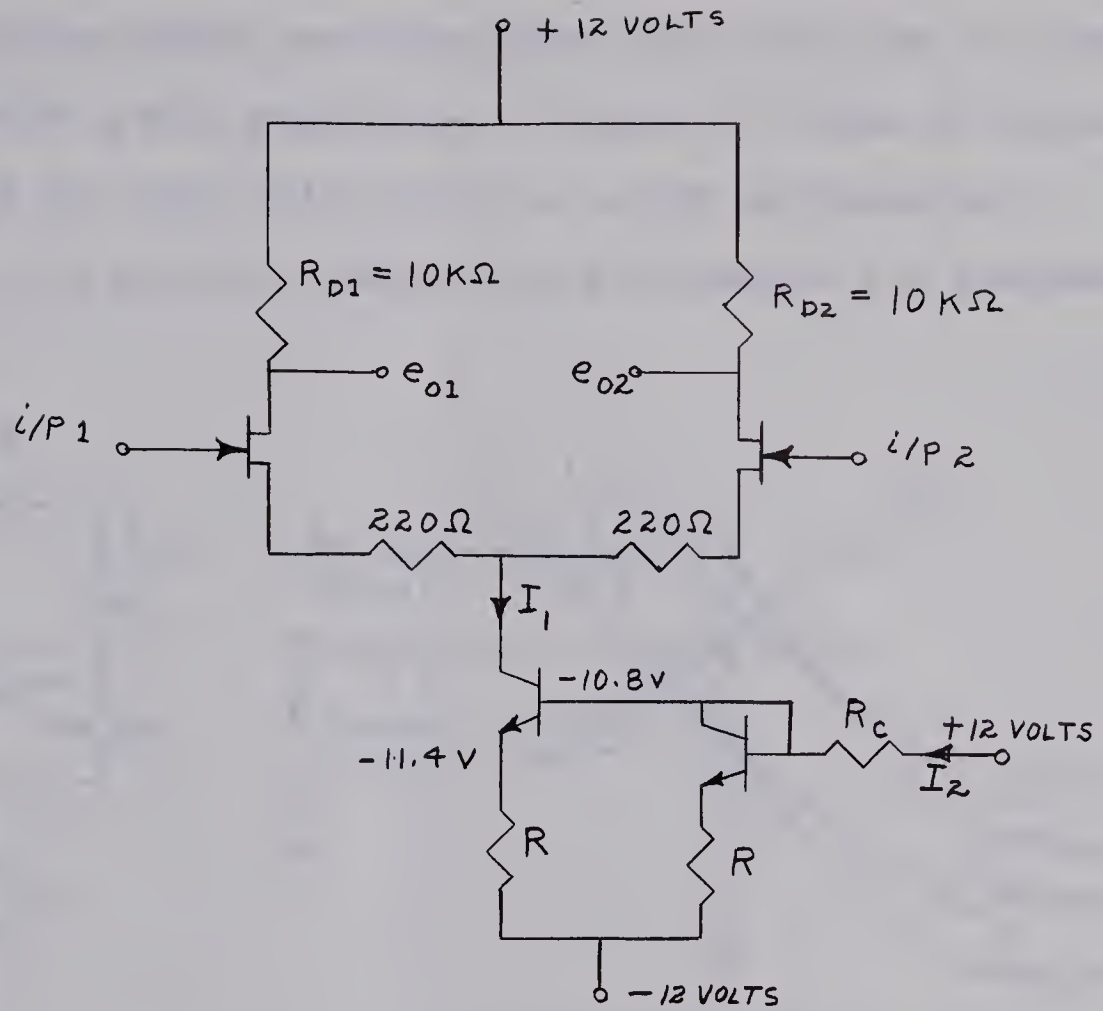


Figure 3.5

Circuit Diagram for FET Differential Amplifier

The following equations were solved to find the values of R and R_C .

$$I_1 = I_2 \quad (3.7)$$

$$I_2 = 1.6 \text{ ma} = \frac{24 - V_{BE}}{R + R_C} = \frac{23.4}{R + R_C} \quad (3.8)$$

$$R_C = \frac{12 - (-10.8)}{I_2} = \frac{22.8}{1.6} \times 10^3 = 14.5 \text{ K}\Omega \quad (3.9)$$

Hence, $R = 375 \Omega$.

The circuit of Figure 3.5 must be modified slightly as in the method outlined by Freyling⁹ to compensate for

the temperature drift resulting from the inability to locate the zero drift point precisely. Figure 3.6 shows an expanded view around the zero drift point of a FET differential amplifier with greatly exaggerated differences for purposes of clarity.

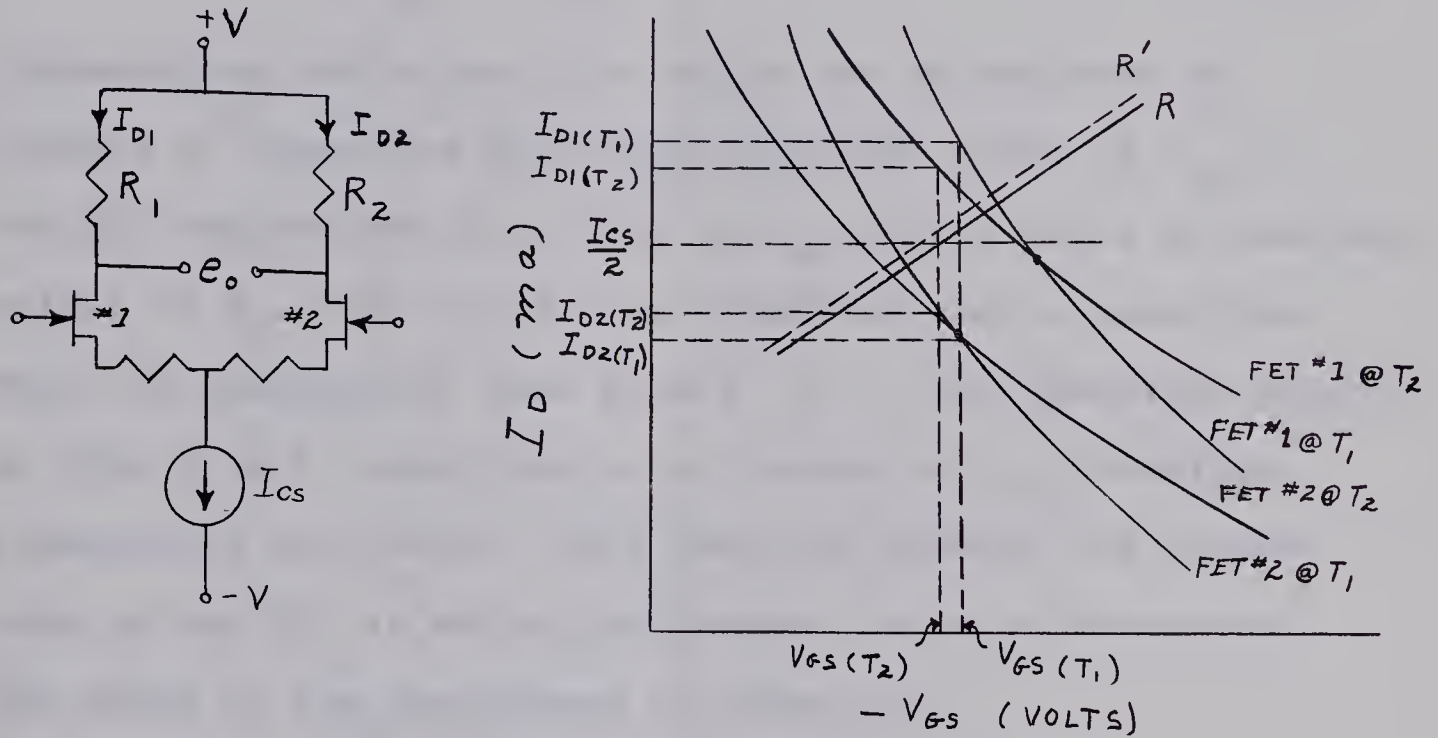


Figure 3.6

Temperature Compensation

Assume that the gate-to-source voltage of the two FET's are equal. Then at Temperature T_1 , the value of $V_{GS}(T_1)$ will be established by the circuit such that

$$I_{D1} + I_{D2} = I_{CS} \quad (3.10)$$

At temperature T_2 , V_{GS} will shift to a new value, $V_{GS}(T_2)$ so that equation 3.10 still holds true. This change will result in a drift at the output since I_{D1} decreased and I_{D2} increased.

Zero drift at the amplifier output, e_o , is defined by

$$\Delta I_{D1} R_1 = \Delta I_{D2} R_2 \quad (3.11)$$

which, since $R_1 = R_2$, requires that

$$\Delta I_{D1} = \Delta I_{D2} \quad (3.12)$$

Compensating the circuit for drift can be achieved by finding an operating point such that the drift in V_{GS} in one FET compensates the drift in V_{GS} in the other at constant values of I_D . The solid line R defines such a condition. When the temperature goes from T_1 to T_2 the operating points go from R to R' resulting in no change in I_D , therefore eliminating any drift. This resistor goes in the source lead of the FET in which the current is to be decreased. The value of the resistance is given by

$$R = \frac{V_{GS}(T_1) - V_{GS}(T_2)}{I_D} \quad (3.13)$$

Although this is a simple solution in theory, in practice one cannot obtain the transfer characteristics of the FET's for two different temperatures with sufficient accuracy to determine this resistance. The transfer characteristics, however, will indicate which source lead should have the extra resistance. From this point, the solution requires a trial and error technique, measuring drift for various values of resistance. Figure 3.7 shows the drift for the final solution of $R = 36 \, \Omega$ for the FET's used in the potential control amplifier. All first order

effects of temperature drift appear to be eliminated leaving a maximum drift of approximately $10 \mu\text{V}/^\circ\text{C}$. The addition of the resistance results in a slight offset voltage at the

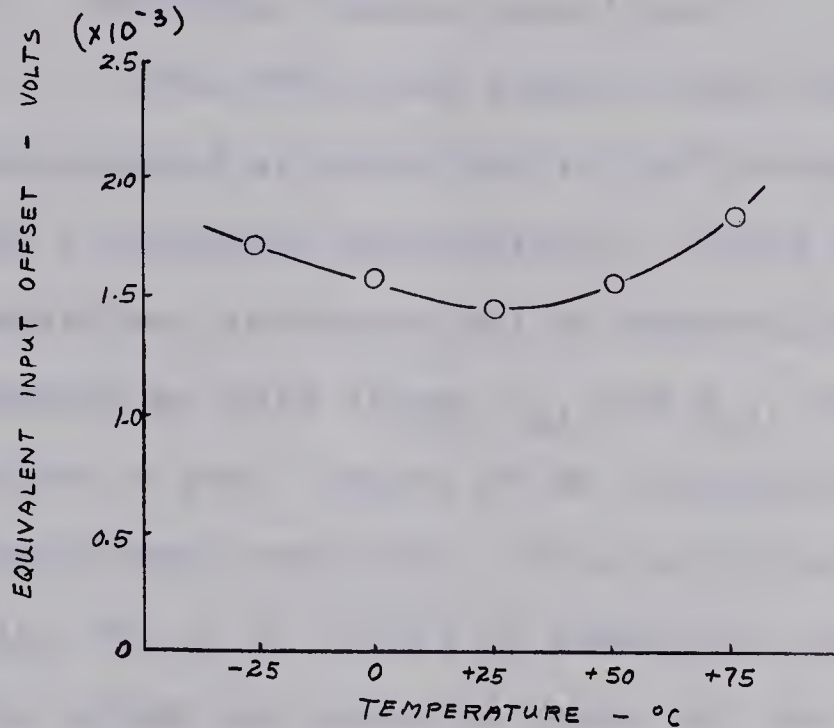


Figure 3.7

Temperature Drift of I/P Stage of Potential Control Amplifier

amplifier output, e_o , as indicated in Figure 3.7. This voltage can be reduced to zero by shunting the load resistor, R_1 or R_2 , through which the most current is flowing with another larger resistor chosen in size such that $e_o = 0$. The slight change in load resistance resulting from the addition of a shunting resistor produces a negligible effect on the overall circuit operation.

The FET input stages of the other two amplifiers, the current and potential measuring amplifiers, were designed in exactly the same manner. The specifications required for these amplifiers were achieved and in most cases exceeded.

3. Complete Electronic Circuits Design

(a) Voltage Clamping System

(i) Potential Control Amplifier

The FET input stage of this amplifier was constructed as described in the previous section and illustrated schematically, along with the total amplifier, in Figure A.1 of Appendix A. The two outputs of this stage, e_{o1} and e_{o2} , were connected to the + and - inputs of an integrated circuit (IC) operational amplifier. This amplifier had a fixed gain of 0.5. A 0.00168 μf capacitor connected between its output and negative input set the cut-off frequency of the amplifier to 2000 KHz. The output of this amplifier was connected to the negative input of another IC operational amplifier. This second IC amplifier provided gain control for the complete amplifier between 0 and 1400 by having a variable resistor in its feedback path. A further D.C. input could be switched into the summing junction of the last IC amplifier's negative input to allow adjustment of the D.C. level of the output. Both IC amplifiers were compensated according to the specifications by their manufacturer. A resistance of 56 Ω was placed directly at the output of each IC amplifier to isolate the operational amplifier from capacitive loads at high frequencies. The total

amplifier output was set to zero, for a zero input, by means of a D.C. biasing arrangement connection to the negative input of the first IC amplifier.

Before deciding on the sizes of the input and feedback resistors on the second IC amplifier, the gain of the input FET stage had to be calculated. Consider the circuit of Figure 3.8 showing the left half of the symmetrical differential amplifier.

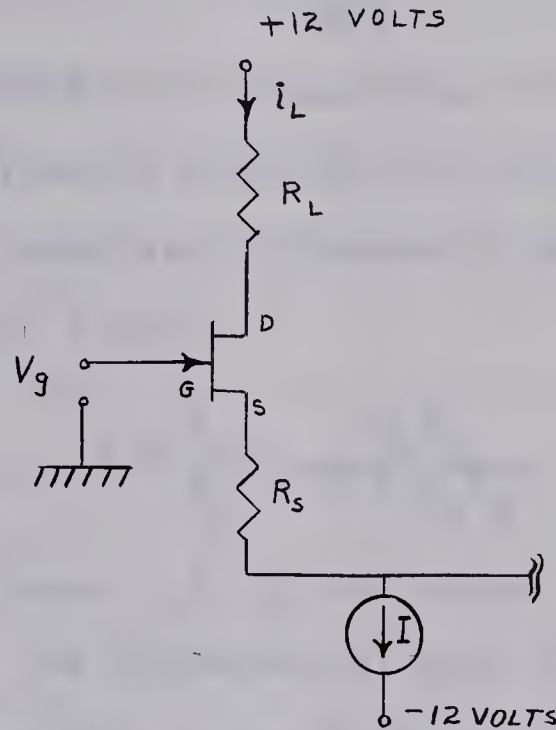


Figure 3.8

One Side of the Differential FET Amplifier

$$i_L = -g_m V_{gs} \frac{1}{1 + g_{DS} (R_L + R_S)} \quad (3.14)$$

where g_{DS} = the drain to source conductance in the pinch-off region.

$$V_{gs} = V_g + i_L R_S \quad (3.15)$$

$$\text{Hence, } i_L = \frac{-g_m (V_g + i_L R_S)}{1 + g_{DS} (R_L + R_S)} \quad (3.16)$$

Rearranging equation 3.16,

$$i_L = \frac{-g_m V_g}{1 + g_{DS} (R_L + R_S) + g_m R_S} \quad (3.17)$$

The value g_{DS} is the slope of the output characteristic in the pinch-off region and is generally small compared to the load conductances. Therefore,

$$i_L \approx \frac{-g_m V_g}{1 + g_m R_S} \quad (3.18)$$

The output of the amplifier is $i_L R_L$. Multiplying both sides of equation 3.18 by R_L gives the gain of the complete differential pair as shown in equation 3.19.

$$A = \frac{V_O}{V_g} \approx \frac{-g_m R_L}{(1 + g_m R_S)} \quad (3.19)$$

From Figure 3.3, g_m was found to be 2.94×10^{-3} mhos. Hence, the differential gain of the FET stage for this amplifier came to approximately 16.8. The gain of Amplifier 1 (see Figure A.1) was set to be 0.5. By selecting the size of the input resistor to Amplifier 2, $R_{in} = 1.5 \text{ K}\Omega$, and the variable feedback resistor of that amplifier, $R_f = 250 \text{ K}\Omega$, a total gain adjustable between 0 and 1400 was achieved.

For later calculations, the frequency response of the potential control amplifier was measured in the laboratory. The results are plotted on Figure A.2.

(ii) Potential Measuring Amplifier

The potential measuring amplifier was designed with a FET input stage as previously described. The complete amplifier schematic is shown in Figure A.3. Only one IC operational amplifier was used in this case since no variable gain was required. The two outputs from the FET stage were connected to the + and - inputs of the IC amplifier as before. The feedback resistors of the IC amplifier were calculated to give a total amplifier gain of approximately 10. R^* was added later when the amplifier was experimentally calibrated to a gain of 10. The gain of the FET input stage was 12.3. As before the IC amplifier was compensated as suggested by the manufacturer. The output was set to zero input by a D.C. offset voltage applied to the negative input terminal of the IC amplifier.

(iii) Current Measuring Amplifier

This amplifier was designed in exactly the same manner as the potential measurement amplifier except the total amplifier gain in this case was set to 100. A 25 pf capacitor was connected between the output and the negative input of the IC operational amplifier to prevent overshoot. The amplifier schematic appears in Figure A.4.

(iv) Clamping Amplifier

The clamping amplifier does not require nearly the input impedance of the other three amplifiers. Consequently, it was possible to use IC operational amplifiers without any FET input stage. The final circuit was constructed as shown schematically in Figure A.5.

The amplifier required a gain variable from 0 to 100. It was not feasible to use the differential input on one IC amplifier and have a variable gain on it at the same time. To achieve the required differential input, two inputs were added at a summing junction, one inverted with respect to the other by another operational amplifier. The summing junction was then connected to the negative input terminal of an IC operational amplifier. The feedback resistor on this amplifier was variable, thus providing the variable gain. A capacitor of 150 pf was placed in parallel with the variable resistor to prevent overshoot of the amplifier for a step input.

A small amount of phase lag occurred in the positive terminal due to the operational amplifier inverting this input. To compensate for this the negative input had to be provided with the same amount of lag. This was done by dividing the input resistor into two components and connecting the

interval between the two to ground through a $.001\ \mu\text{f}$ capacitor. This amplifier proved satisfactory in operation after extensive laboratory testing. The frequency response was determined experimentally and is plotted in Figure A.6.

(b) Voltage Generator System

As previously mentioned, this system was constructed to provide stimulating and clamping pulses to the nerve fibres. The system was composed of seven parts:

1. Trigger Circuit
2. Monostable Multivibrator
3. Logic Patchboard
4. D/A Converter
5. Inverter (Operational Amplifier)
6. Gate
7. Power Supply

The first six parts were connected together to form the total system as shown in Figure A.7. The power supply used for this system, as well as the clamping system, was designed by Mr. E. M. Edwards and was constructed for use in the Electrical Engineering laboratories at this University.

(i) Trigger Circuit

The first part of the trigger circuit, as shown in Figure A.8, employs a unijunction transistor (UJT) as the oscillating device. The output

of the UJT is a train of spikes, the period of which is set by the combination of R_1 or R_2 and C_1 , C_2 or C_3 . By making R_1 and R_2 variable and installing switches as indicated in the schematic, three frequency ranges were obtained:

1. 0.01 Hz - 12 Hz

2. 12 Hz - 130 Hz

3. 60 Hz - 5000 Hz

These three ranges provided a more than adequate frequency capability for the voltage generator system.

The last two transistors shown in Figure A.8 were used to shape the spike output of the UJT oscillator into a train of squared pulses of an amplitude of approximately 3 volts. The 880 pf capacitor to ground at the output of the circuit eliminated any overshoot or spikes in the output waveform. Due to the 12 volt spike output of the UJT oscillator, care had to be taken throughout the design of the total system to guard against spikes occurring in the output waveform of the system. This possibility could occur through either capacitive coupling between circuits or spikes carried through on the ground or power supply bus bars.

(ii) Monostable Multivibrator Circuit

The output signal of the trigger circuit was connected to the logic patchboard on which it

passed through a Schmitt trigger and two gates to permit, by the use of other logic devices, control of the voltage generator system. The nature of this control feature will be discussed later. From the logic patchboard the triggering signal was fed into the input of a monostable multivibrator, the circuit schematic of which is found in Figure A.9.

In the stable state Q_2 is held in the 'on' state by the base current through R_3 . Q_1 is held in the 'off' state by the -12 volt supply, R_1 and R_2 . Triggering the base of Q_1 will cause the circuit to change to its astable state (Q_1 'on' and Q_2 'off') which will exist for a time determined by R_3C_4 or R_3C_5 . When C_4 or C_5 has changed such that Q_2 begins to turn on, Q_1 turns off and the cycle is complete. The circuit will then remain in the stable state until another trigger pulse is fed to the base of Q_1 .

The output of the circuit will be a pulse whose width is determined by R_3C_4 or R_3C_5 depending on the position of switch, S_1 . The two capacitors, C_4 and C_5 , and switch, S_1 , allow one to switch between a short variable duration pulse used for stimulating the fibres and a very much longer variable duration pulse used for clamping the fibres. The ranges of these two states were set to the following:

Stimulating pulse: 50 μ sec to 4 msec

Clamping pulse: 4 msec to 400 msec

Transistors Q_3 and Q_4 were added to the circuit for two purposes. The first was to provide a pulse with a fast risetime by using the output of transistor Q_2 . The addition of Q_3 allowed Q_4 to be turned 'on' during the time that Q_2 was 'off', i.e. when the circuit was in the astable state. The other purpose was to permit control of the amplitude of the voltage at the output of the circuit. The emitter of Q_4 was connected to a switch which could select one of two inputs. One of these inputs was the voltage whose amplitude was automatically controlled by the logic patchboard and a Digital/Analog (D/A) converter and the other was the voltage whose magnitude was controlled manually by means of a potentiometer. The capacitor from the collector of Q_4 to ground was again added to prevent fast spikes from occurring in the output.

(iii) Gate Circuit

The gate was used primarily as a means of providing a low output impedance for the voltage generator system. It also provided a means of selecting a positive or negative output by the position of switch, S_1 .

The gate, as shown in Figure A.10, was

constructed from two operational amplifiers. Those amplifiers, OA3's, were designed by Mr. E. M. Edwards and constructed in the Electrical Engineering Department at this University. R_1 was used to calibrate the output such that the automatic sequencing of pulse amplitudes occurred with increments of 5, 10, 20 or 40 mv. The output of the first operational amplifier provided a negative pulse. Another amplifier was added to invert the negative pulse, thus supplying the positive pulse output. The output impedance of either amplifier was approximately 60 Ω . Elimination of the D.C. offset voltage at the output with the input grounded was achieved as before by supplying a small D.C. offset voltage at the negative input terminal of each operational amplifier.

(iv) Logic Patchboard

The logic patchboard, made up of IC flip-flops, gates, buffers and Schmitt triggers, was designed, as well, by Mr. E. M. Edwards. All the input and output terminals of each IC were brought to a surface patchboard where they could be interconnected with plug-in wires.

The logic system was designed to be used primarily for the clamping portion of the voltage generator system. By means of a counter, made up

of seven JK flip-flops, connected to a D/A converter, a series of long duration pulses could be obtained with each pulse being 5, 10, 20 or 40 mv greater in amplitude than the previous. In this way the voltage generator system could be used to display on a storage oscilloscope clamped responses to several pulses whose amplitude could be selected in the increments indicated above. Gates were used to stop the counting sequence, as well as any output from the voltage generator, when the maximum count was reached. The count at which the system was to be stopped could be selected by patching the gate inputs to either the Q or $\bar{Q}$ outputs of the flip-flops. A 'HALT' switch was installed to manually stop the function of the generator at any time. A 'RESET' switch was installed to reset the logic after it had been stopped by the gates connected to the flip-flops. The gates associated with the 'RESET' and 'HALT' pushbutton microswitches were used to prevent multiple signals due to bouncing of the contact points when they came together in the switches. Indicator lamps were used to show, digitally, at what point in the counting sequence the system was at any given time.

Figure A.11 shows the connections which were made to allow the counter to go from 1 to 64 before it was stopped. The output of the trigger

circuit was fed into the Schmitt trigger which is shown at the bottom of the figure. The gates at the output of the Schmitt trigger were used to prevent the trigger pulse from passing through to the monostable multivibrator when the counter had stopped. This, of course, could be by-passed merely by unplugging the top wire in the first gate following the Schmitt trigger.

(v) D/A Converter and Inverter Operational Amplifier

This circuit, designed by Mr. E. M. Edwards, converts a digital number obtained from the counter on the logic patchboard into its equivalent analog signal. With the logic connections as indicated in Figure A.11, the voltage amplitude would start at zero volts and would increment by -5 mv each time the trigger circuit fired. It would do this 64 times reaching a maximum value of -320 mv. The voltage range and the increment size could be changed quite easily by changing a few of the logic connections.

The output of the D/A converter was negative and since a positive output was required an operational amplifier was used to invert the signal. This amplifier was the same as those used in the gate circuit. Figure A.12 shows the component sizes used for this particular application.

(vi) Total Voltage Generator System Operation

By using logic to control the amplitude of the output voltage and, as well, providing a manual override, this system is quite versatile. The logic connections could be changed such that the amplitude of the output pulses could be started and stopped at any point in the sequence. If automatic control of the amplitude was not desired the manual control could be used to set it to any value and hold it constant at that value. The trigger and monostable multivibrator circuits provided for any frequency and pulse duration which would be required in conducting clamp experiments on nerve fibres. The output could be chosen to be either positive or negative for both the manual and automatic mode.

The system was tested thoroughly in the laboratory to insure all specifications were met. Initially problems arose due to spikes occurring on the output waveform. Despite the difficulty in locating the source of these spikes they were eventually eliminated and the operation of the system was considered completely satisfactory.

B. Mathematical Analysis of System Performance

1. Sources of Error in Measuring System

As with all the clamping techniques described in the introduction, there are a few slight errors inherent in this

technique. One of the errors, associated with the potential control system, was discussed previously in the description of this clamping technique in the Introduction. This error occurred as a result of the feedback amplifier, with a gain of 1000, maintaining pool A (see Figure 1.1) at a potential 99.5 per cent of the true transmembrane potential. An error of 1/2 per cent is insignificant considering that the equivalent electrical parameters of the nerve fibre cannot be determined for analytical purposes to anything approaching that accuracy.

Another slight error is introduced by the fact that the measuring amplifiers have a finite input impedance and input offset current. The system was designed so that the error caused by this effect was limited to a few millivolts at worst. This, again, is well within the accuracy with which the system can be treated analytically. The system could be designed even more stringently to decrease this to less than 1 mv; however, it was felt that the limited ability to estimate the system parameters for analytical purposes did not warrant this extreme accuracy.

The major problem in using this technique for clamping purposes concerned the stability of the negative feedback potential control system and, to a lesser degree, the stability of the clamping system itself. The stability of both these systems were examined mathematically to determine the system's performance under various conditions.

Other problems were encountered in the experiments

carried out using this system. These will be discussed later along with the experimental results.

2. Approaches to System Stability Analysis

Two methods of analysis were considered. The first was to treat the system precisely as is, deriving complex differential equations to describe the nerve fibre and feedback amplifiers in the time domain. Then the numerous equations necessary to describe the system would have to be solved in the time domain by use of numerical methods. For the description of the voltage waveform moving down the internodal region between nodes N_2 and N_3 only, this would involve solving in excess of 100 simultaneous differential equations.^{10, 11} The results by this sort of approach, if attainable, would probably be put in the form of an impulse or step response and a Bode Plot. From these forms the stability could then be examined.

By making a few assumptions, which can be easily justified when one considers the complexity of a solution of the above form, a solution can be carried out in the frequency domain. This solution, the alternative to the above, can also be put in the form of a Bode Plot and a step response.

The nature of the system prevented one from solving the system's transfer function for the roots. Therefore, the poles and zeros of the system were not readily available to be used in the more conventional approach to stability

analysis. The reason for this, as will be seen later, is due to an irrational transfer function for the nerve fibre itself.

The open loop transfer function for the system was derived; however, it did not prove to be very useful in the stability analysis in its open loop form. Consequently, the transfer function for the closed loop system was computed and a Bode Plot and step response plotted out. The Bode Plot would indicate the location of poles to the right of the imaginary axis on a complex plane (and thus instability) by showing the magnitude rising to infinity at the location of the pole. Location of poles directly on the imaginary axis would not be indicated on the Bode Plot.

In order to determine whether poles did indeed exist on the imaginary axis, a step response was computed for both the potential system and the clamping system. If poles existed on the imaginary axis, sustained oscillations would be evident in the step response.

In order to begin the solution of the problem by the second approach outlined above it was, of course, necessary to set the problem up mathematically. This required a representation of the nerve fibre by means of an equivalent circuit showing the points at which the amplifiers made contact with the nerve. Then it was necessary to derive a transfer function, or impedance function, for the nerve fibre itself. Having done this,

and having determined the frequency characteristics of the amplifiers, a Bode Plot was prepared first of all for the potential control system only. A step response for this system was then carried out for various gains.

A study was also made to determine the relation between the maximum values of the magnitude part of the Bode Plot and gain. Also the relation between the frequencies at which those maximums occurred and gain was determined. The Bode Plots and step responses were then calculated for the complete clamping system.

The calculations were programmed in the APL language and carried out on the University of Alberta IBM 360/67 computer system. The computer was programmed to output the data in the forms of the desired plots, i.e. Bode Plots and step responses. Thus the graphs appearing in the results of these calculations are actual direct computer printouts.

As previously mentioned, it was necessary to make a few assumptions before this approach to stability analysis could be taken. These assumptions, with a discussion of the possible error they could introduce, appear in the next section.

3. Assumptions In Stability Analysis Calculations

Most of the assumptions involve the derivation of the Laplace transform of the nerve fibre impedance function. The remaining portion of the system, i.e. the amplifiers and

electrodes, could be easily handled by Laplace transform methods without requiring any assumptions to simplify the calculations. The assumptions, with an estimate on error introduced by each, are listed below.

(a) The nerve fibre was assumed to be a uniform cable like structure going from $-\infty$ to $+\infty$ with a conduction core and surface membrane having resistive and capacitive properties. For the purposes of the discussion on the calculations the nerve fibre will now be referred to as a cable.

This assumption results in no error in itself but merely provides a basis for the derivation of the cable transfer function. The cable was sufficiently long such that the effects occurring in the two internodes in the clamp apparatus behaved precisely as if the cable did extend from $-\infty$ to $+\infty$.

(b) The current leakage to ground was assumed to be very much less than the axial current. As a result of this assumption all the current flowing into the nerve fibre at one end was assumed to appear at the other end. In actual fact a small amount of current flowed from inside the axon through the myelin sheath to the grounded pool B. The resistance through the myelin sheath was sufficiently high that this current was limited to a small amount. Only about 1/4 of the internodal region appeared in the grounded pool. Calculations indicate that this could result in a possible error in axial current of

approximately 2.5 per cent.

(c) The entire internode between node N_2 and N_3 was assumed to be in pool B which was at ground potential. Since all current to ground was disregarded by the previous assumption, this assumption would not introduce an error in the axial current. This assumption allowed one to treat the internode as a separate unit with one input and one output.

(d) All the current passing through the internode between nodes N_2 and N_3 was assumed to pass through the node N_3 . This is not exactly true in the actual case. The membrane resistances at the nodes in the end pools were reduced to 1-4 $M\Omega$ by depolarization. Consequently, this impedance is about 1/25 that of the next internode so that the error in the terminating conditions at the node N_3 is about 4 per cent. This, in turn, will result in an error less than 1 per cent in the axial current.

(e) The intracellular resistance was assumed to be entirely ohmic. This is a valid assumption to make and no error is expected to result from it.

(f) The membrane capacity was assumed to be a pure capacitor in parallel with the membrane. This assumption stems from the equivalent circuit of the nerve fibre. The equivalent circuit must necessarily regard its components as pure elements.

(g) The extracellular fluid was assumed to be of sufficient volume to have a negligible resistance and to be

virtually isopotential. This, of course, is not exactly true; however, the impedance effect due to the extra-cellular fluid would be negligible in comparison to that of the impedances in the various parts of the circuit. The error resulting from this assumption was indeterminant.

(h) One last assumption involved the non-linearities of the system. The non-linearities of the system were ignored since it was felt that the system had to go unstable, if it did at all, in the linear portion of the system. Non-linearities of the system occurred in the transmembrane voltage generator where the action potential occurred due to a constant voltage and an altering resistance. For the purpose of this analysis this assumption considered the resistance as constant and the voltage as changing. A limited amount of error is expected to be involved with this assumption; however, its magnitude is difficult to assess.

4. Derivation of Cable Transfer Function

Consider the cable shown in Figure 3.9.

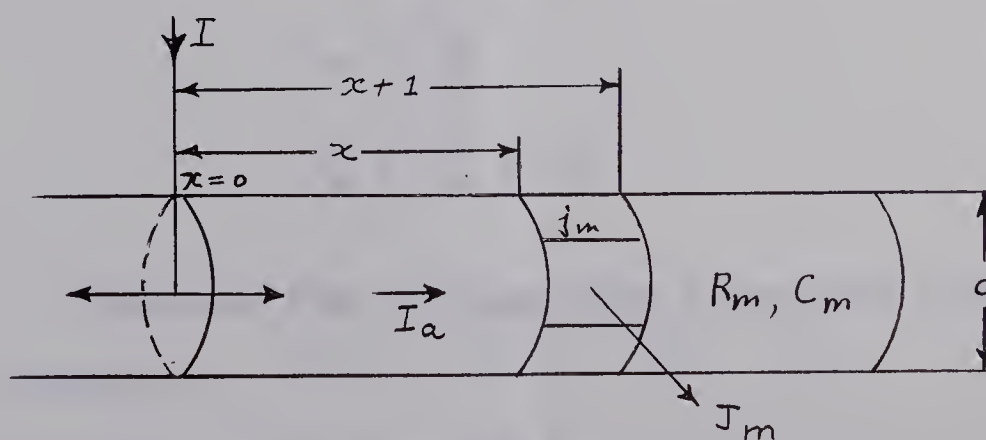


Figure 3.9

Cable Representation of Nerve Fibre

Definitions of parameters are as follows:

x = distance along axis (cm)

d = cable diameter (cm)

t = time (msec)

V = potential of intracellular fluid as a derivative from the quiescent potential (mv)

r_a = resistance/unit length of axoplasm (Ω/cm)

ρ = resistivity of intracellular fluid ($\Omega \text{ cm}$)

r_m = resistance of membrane in a unit length of axon ($\Omega \text{ cm}$)

R_m = resistance of unit area of membrane ($\Omega \text{ cm}^2$)

C_m = capacity of unit area of membrane ($\mu\text{f}/\text{cm}^2$)

I_a = axial current (μA)

j_m = current density crossing membrane at a given point referred to a unit of axon ($\mu\text{A}/\text{cm}$)

J_m = current density crossing membrane at a given point referred to a unit area of membrane ($\mu\text{A}/\text{cm}^2$)

J = current density applied from external source

I = current applied from external source

$$r_a = \frac{4\rho}{\pi d^2} \quad (3.20)$$

$$r_m = \frac{R_m}{\pi d} \quad (3.21)$$

$$j_m = J_m \times \pi d \quad (3.22)$$

Consider the voltage drop along the cable due to axial current.

$$\Delta V = -I_a r_a \Delta x \quad (3.23)$$

or as $\Delta x \rightarrow 0$;

$$\frac{\partial V}{\partial x} = -I_a r_a \quad (3.24)$$

Similarly the current drop along the cable is defined as

$$\frac{\partial I_a}{\partial x} = -j_m \quad (3.25)$$

Differentiating equation 3.24 and substituting equation 3.20 and 3.25,

$$J_m = \frac{d}{4\rho} \frac{\partial^2 V}{\partial x^2} \quad (3.26)$$

The membrane current has three components: a capacitive component charging up membrane capacity; an ionic component, J_i , flowing across the membrane; and an external component, J . Therefore,

$$\frac{d}{4\rho} \frac{\partial^2 V}{\partial x^2} = C_m \frac{\partial V}{\partial t} + J_i + J \quad (3.27)$$

Consider a linear cable.

$$J = 0 \quad (3.28)$$

$$J_i = \frac{V}{R_m} \quad (3.29)$$

Hence,

$$\frac{d}{4\rho} \frac{\partial^2 V}{\partial x^2} = C_m \frac{\partial V}{\partial t} + \frac{V}{R_m} \quad (3.30)$$

This equation holds true at all points except $x = 0$. The boundary conditions at $x = 0$ will be incorporated later.

Taking the Laplace transform of equation 3.30 gives

$$\frac{d^2V}{dx^2} - K_1 \left(S + \frac{1}{\tau} \right) V = 0 \quad , \quad (3.31)$$

where $K_1 = \frac{4\rho C_m}{d}$

and $\tau = R_m C_m$.

The differential equation 3.31 can now be solved.

Let $P = \frac{dV}{dx}$,

and $\frac{d^2V}{dx^2} = \frac{dP}{dx} = \frac{dP}{dV} \cdot \frac{dV}{dx} = P \frac{dP}{dV}$. (3.32)

Substituting into equation 3.31,

$$P \frac{dP}{dV} - K_1 \left(S + \frac{1}{\tau} \right) V = 0 \quad (3.33)$$

$$P dP - K_1 \left(S + \frac{1}{\tau} \right) V dV = 0 \quad . \quad (3.34)$$

Integrating,

$$\frac{P^2}{2} - K_1 \left(S + \frac{1}{\tau} \right) \frac{V^2}{2} = \frac{C_1^2}{2} \quad (3.35)$$

Where C_1 = an arbitrary constant.

Then, $P = \frac{dV}{dx} = \pm \sqrt{C_1^2 + K_1 \left(S + \frac{1}{\tau} \right) V^2}$ (3.36)

Rearranging equation 3.36,

$$\pm dx \sqrt{K_1 \left(S + \frac{1}{\tau} \right)} = \frac{dV}{\sqrt{\frac{C_1^2}{K_1 \left(S + \frac{1}{\tau} \right)} + V^2}} \quad (3.37)$$

Integrating equation 3.37,

$$\pm (C_2 + x) \sqrt{K_1 \left(S + \frac{1}{\tau}\right)} = \ln \left[V + \sqrt{\frac{C_1^2}{K_1 \left(S + \frac{1}{\tau}\right)} + V^2} \right]. \quad (3.38)$$

Rearranging equation 3.38,

$$V + \sqrt{\frac{C_1^2}{K_1 \left(S + \frac{1}{\tau}\right)} + V^2} = e^{\pm C_2 \sqrt{K_1 \left(S + \frac{1}{\tau}\right)}} e^{\pm x \sqrt{K_1 \left(S + \frac{1}{\tau}\right)}}. \quad (3.39)$$

The following boundary conditions can now be incorporated:

When $x = \infty$, $V = 0$.

For this to be true, the exponents of e have to be negative.

Equation 3.39 then becomes

$$V + \sqrt{\frac{C_1^2}{K_1 \left(S + \frac{1}{\tau}\right)} + V^2} = 0. \quad (3.40)$$

Solving equation 3.40, the constant C_1 is found to be equal to zero.

Hence equation 3.39 becomes

$$V = \frac{1}{2} \cdot e^{-C_2 \sqrt{K_1 \left(S + \frac{1}{\tau}\right)}} \cdot e^{-x \sqrt{K_1 \left(S + \frac{1}{\tau}\right)}}. \quad (3.41)$$

Consider the boundary conditions at $x = 0$.

$$\text{When } x = 0, I_a = \frac{I}{2}. \quad (3.42)$$

$$\text{Let } A = \frac{1}{2} \cdot e^{-C_2 \sqrt{K_1 (S + \frac{1}{\tau})}}. \quad (3.43)$$

Then, differentiating equation 3.41,

$$\frac{dV}{dx} = -A \sqrt{K_1 (S + \frac{1}{\tau})} \cdot e^{-x \sqrt{K_1 (S + \frac{1}{\tau})}}. \quad (3.44)$$

Substituting in equation 3.24,

$$I_a = \frac{A \sqrt{K_1 (S + \frac{1}{\tau})}}{r_a} \cdot e^{-x \sqrt{K_1 (S + \frac{1}{\tau})}}. \quad (3.45)$$

At $x = 0$ and since $I_a = \frac{I}{2}$, equation 3.45 becomes

$$A = \frac{I r_a}{2 \sqrt{K_1 (S + \frac{1}{\tau})}}. \quad (3.46)$$

Substituting into equation 3.45, the final result is achieved.

$$L(s) = \frac{V(s)}{I(s)} = \frac{r_a}{2 \sqrt{K_1 (S + \frac{1}{\tau})}} \cdot e^{-x \sqrt{K_1 (S + \frac{1}{\tau})}} \quad (3.47)$$

Equation 3.47 is essentially an impedance function and was used as such in the stability calculations.

5. Potential Control System Stability

Figure 3.10 shows a representation of the transmembrane potential control system.

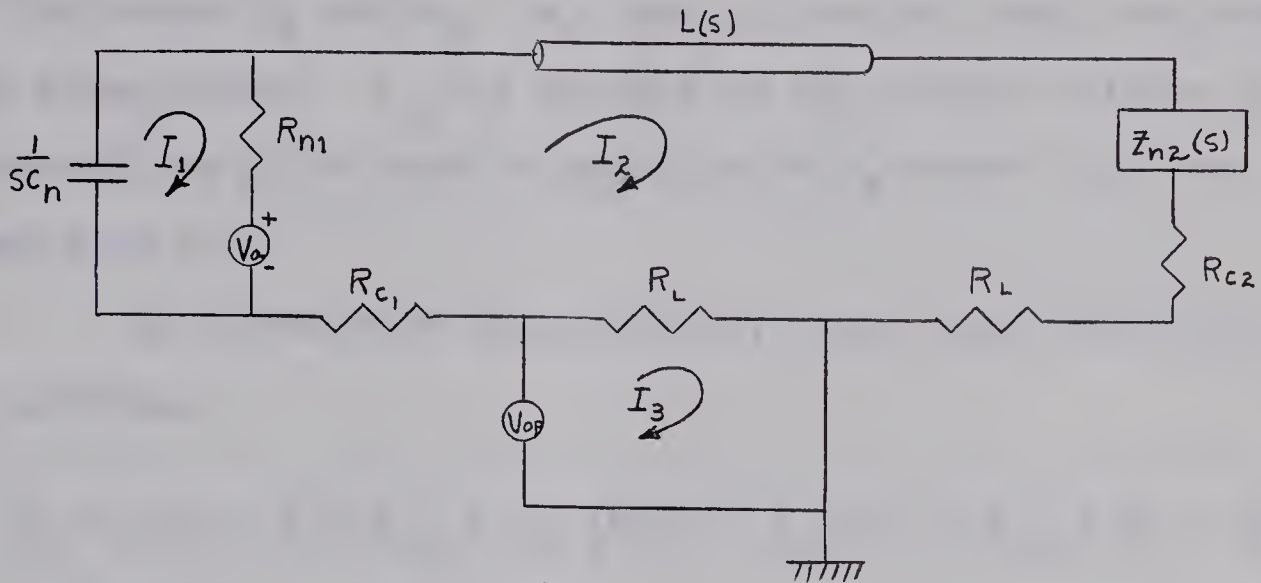


Figure 3.10

Transmembrane Potential Control System

The cable between nodes N_1 and N_2 was disregarded for purposes of this calculation. V_{op} would supply any current necessary to approximate $-V_m$. This current, half of which goes in either direction from the node N_2 would result in a slight error since it all must pass through R_{n1} . Due to the small current under controlled conditions this error was considered negligible in comparison to those resulting from the assumptions.

In Figure 3.10, $L(s)$ is the cable impedance function previously derived. $Z_{n2}(s)$ is the nodal impedance at the depolarized node, N_3 . R_L is the resistance across the external petroleum jelly seals. It is formed from an annular region filled with electrolytic fluid bounded by the fibre itself and the petroleum jelly placed around it.

The value of this resistance depends on how tightly the petroleum can be packed around the fibre. R_{c1} and R_{c2} are the convergence resistances going into the exposed membrane at the nodes N_2 and N_3 . R_{n1} and C_n are the nodal resistance and capacitance. V_{op} is defined as the output voltage of the feedback amplifier and is equal to KI_2R_L where K is the amplifier gain.

By Kirchoff's voltage laws, three loop equations can be written.

$$V_a = (I_2 - I_1) R_{n1} + I_2 \left[L(s) + Z_{n2}(s) + R_{c2} + R_L + R_{c1} \right] + (I_2 - I_3) R_L \quad (3.48)$$

$$V_{op} = (I_3 - I_2) R_L = KI_2 R_L \quad (3.49)$$

$$V_a = -I_1 \left(R_{n1} + \frac{1}{sC_n} \right) + I_2 R_{n1} \quad (3.50)$$

From equation 3.49,

$$I_3 = (K + 1) I_2 \quad (3.51)$$

From equation 3.50,

$$I_1 = \frac{I_2 R_{n1} - V_a}{R_{n1} + \frac{1}{sC_n}} \quad (3.52)$$

Substituting equations 3.51 and 3.52 into equation 3.48 and rearranging,

$$\frac{I_2}{V_a} = \frac{2R_{n1} + \frac{1}{sC_n}}{\left(R_{n1} + \frac{1}{sC_n} \right) \left[L(s) + Z_{n2}(s) + R_{c1} + R_{c2} - (K - 1) R_L \right] + R_{n1} \cdot \frac{1}{sC_n}} \quad (3.53)$$

Since $V_{op} = KI_2 R_L$, with some simplification, equation 3.53 becomes

$$\frac{V_{op}}{V_a} = \frac{2 (SC_n R_{n1} + 1) K R_L}{(SR_{n1} C_n + 1) \left[L(s) + Z_{n2}(s) + R_{c1} + R_{c2} - (K - 1) R_L \right] + R_{n1}} \quad (3.54)$$

The value of K , the potential control amplifier transfer function, was determined from its frequency response, Figure A.2, and is given by

$$K = \frac{-G}{(1 + S\tau_A) (1 + S\tau_B) (1 + S\tau_C) (1 + S\tau_D) (1 + S\tau_E)} \quad (3.55)$$

One further variable to be defined, $Z_{n2}(s)$, is as follows:

$$Z_{n2}(s) = \frac{R_{n2}}{1 + SR_{n2} C_n} \quad (3.56)$$

The transfer function of equation 3.54 was programmed in the APL computer language and the magnitude portion of a Bode Plot was calculated showing the results for various values of gain. Figure B.1 of Appendix B is the resulting plot. The following values were assigned to the variables used in the calculations:

$R_{n1} = 40 \text{ M}\Omega$	$d = 0.001 \text{ cm}$
$R_{n2} = 5 \text{ M}\Omega$	$x = 0.2 \text{ cm}$
$R_m = 0.16 \text{ M}\Omega - \text{cm}^2$	$\tau_A = 7.23 \times 10^{-5} \text{ sec}$
$C_m = 0.004 \text{ }\mu\text{f/cm}^2$	$\tau_B = 5.30 \times 10^{-7} \text{ sec}$
$r_a = 140 \text{ M}\Omega/\text{cm}$	$\tau_C = 2.27 \times 10^{-7} \text{ sec}$
$R_{c1} = 100 \text{ K}\Omega$	$\tau_D = 7.23 \times 10^{-8} \text{ sec}$
$R_{c2} = 100 \text{ K}\Omega$	$\tau_E = 7.23 \times 10^{-8} \text{ sec}$
$R_L = 10 \text{ M}\Omega$	$G = \text{D.C. gain} - \text{variable}$
$\rho = 110 \text{ }\Omega\text{-cm}$	$C_n = 1.5 \text{ pf}$

It is interesting to note that the maximum values of the magnitude change with gain. As mentioned previously, when considering the system in its closed form, any instabilities due to poles to the right of the imaginary axis would appear on the plot as a point of infinite magnitude. It was felt that there was a certain gain where the system could go unstable.

In order to further investigate the apparent dependency of stability on gain, a computer program was written to determine the maximum magnitude for a given gain and, as well, the frequency at which the maximum occurred. Figure B.2 shows the results of the calculations determining the maximum values versus amplifier gain. Figure B.3 shows the frequencies at which these maximums were reached. From Figure B.2, it appears that the system does indeed go unstable at a gain of approximately 300 and oscillates at a frequency of about 350 KHz. Further expansion of the

graphs about these points seems to confirm the instability at a gain of 302 and a frequency of oscillation of 360 KHz.

The next procedure in the calculations was to examine the step response. Since the transfer function for the potential control circuit was in the frequency domain and was too complex to transform into the time domain, it was necessary to calculate the step response itself in the frequency domain and transform the result to the time domain. This was done by using the Fourier Integral pair. The Fourier series for a long duration square wave 1 volt in amplitude was calculated to as many harmonics as the computing system could handle. Each harmonic was represented by a complex number. The period of the square wave was chosen such that all system transients died out long before the voltage waveform reached half its period. The complex numbers representing the harmonics were then multiplied by the value of the system transfer function evaluated at the frequency of the harmonic. The Fourier inverse was then calculated resulting in the required response.

Computer programs were written to carry out these calculations for various values of gain. Figure B.4 shows the voltage V_{op} for a 1 volt input, V_a .

In order to investigate the changes in the waveform as it passed through the system, the time domain response for both the input and output was calculated. Figure B.5 represents the first portion of a square wave input voltage which was calculated from the Fourier series of a square

wave to 60 harmonics. The overshoot and decaying oscillatory behavior at the leading edge are known as the Gibbs effect.¹² As the number of harmonics, n , goes to infinity the overshoot is 9 per cent of the total jump. Also, as n increases, both the frequency of the oscillations and the rate of decay increase. Unfortunately, the number of harmonics which were calculated was limited by the computing facilities. Consequently it was necessary to compare the output to the input rather than assume the input was a true square wave and observe the output only.

6. Voltage Clamp System Stability Analysis

As in the previous case, Kirchoff's voltage laws were employed to derive the total system transfer function. For purposes of calculating the Bode Plot the transfer function derived consisted of the relationship between the input voltage to the potential control amplifier and the clamping voltage. Figure 3.11 shows the system representation for the purpose of the stability analysis.

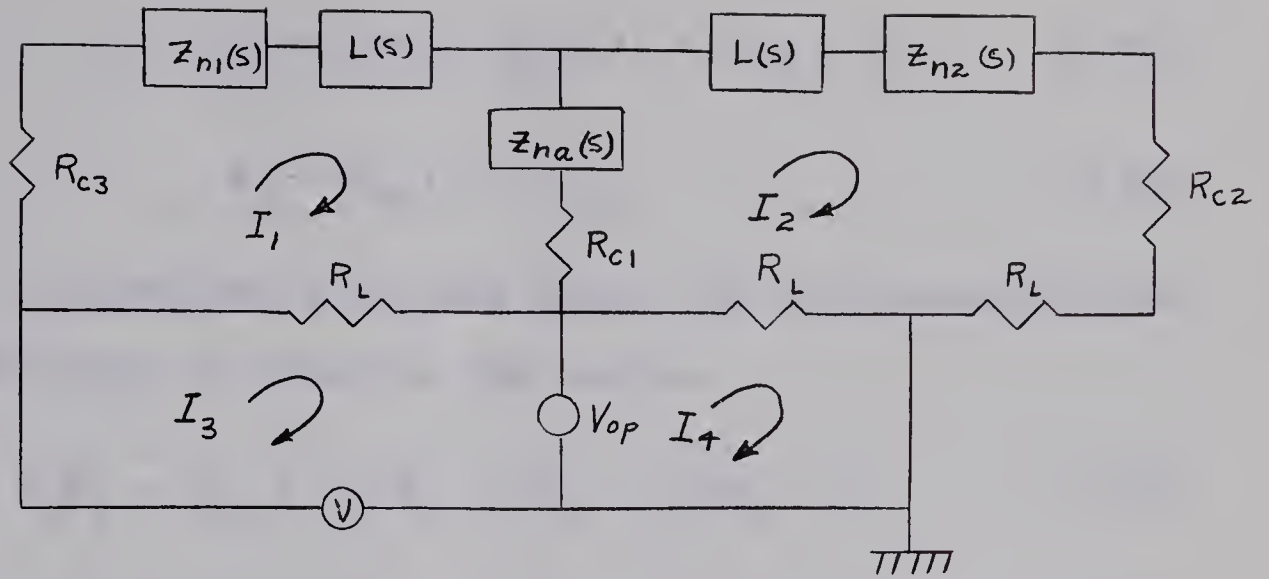


Figure 3.11

Clamping System Representation

R_L , as before, represents the seal resistance.

R_{c1} , R_{c2} and R_{c3} are convergence resistances to nodes N_2 , N_1 and N_3 . $L(s)$ is the cable impedance function previously calculated. The remaining parameters are defined as follows:

$$z_{n1}(s) = \frac{R_{n1}}{1 + sR_{n1}C_{n1}} \quad (3.57)$$

$$z_{n2}(s) = \frac{R_{n2}}{1 + sR_{n2}C_{n2}} \quad (3.58)$$

$$z_{na}(s) = \frac{R_{na}}{1 + sR_{na}C_{n2}} \quad (3.59)$$

$$V_{op} = KI_2 R_L \quad (3.60)$$

$$V = (V_c - V_{op})K_c = K_c(V_c - KI_2 R_L) \quad (3.61)$$

Let

$$z_1 = z_{n1}(s) + L(s) + R_{c3} \quad (3.62)$$

$$Z_2 = L(s) + Z_{n2}(s) + R_{C2} + R_L \quad (3.63)$$

$$Z_3 = Z_{na}(s) + R_{C1} \quad (3.64)$$

By Kirchoff's voltage laws, the loop equations can now be written to describe the system.

$$I_1 Z_1 + (I_1 - I_2) Z_3 + (I_1 - I_3) R_L = 0 \quad (3.65)$$

$$I_2 Z_2 + (I_2 - I_4) R_L + (I_2 - I_1) Z_3 = 0 \quad (3.66)$$

$$V - (I_3 - I_1) R_L - V_{op} = 0 \quad (3.67)$$

$$V_{op} - (I_4 - I_2) R_L = 0 \quad (3.68)$$

One further equation is necessary to solve for the system transfer function. Equation 3.69 describes the output voltage, i.e., the voltage across the input of the potential control feedback amplifier.

$$V_o = I_2 R_L \quad (3.69)$$

Equations 3.65 to 3.69 inclusive can be solved in a manner similar to the potential control system to give the following transfer function.

$$\frac{V_o}{V_c} = \frac{K_C Z_3 R_L}{(Z_1 + Z_3) (Z_2 - K R_L + Z_3) + Z_3 [K R_L (K_C + 1) - Z_3]} \quad (3.70)$$

$$\text{where } K_c = \frac{G_2}{(1 + S\tau_{CA})(1 + S\tau_{CB})} \quad , \quad (3.71)$$

Equation 3.70 describes the clamping amplifier's transfer function and was derived from the frequency response shown in Figure A.6.

The values for the variables used in the equations involved in the transfer function are as follows:

$r_a = 140 \text{ M}\Omega/\text{cm}$	$\rho = 110 \text{ }\Omega\text{-cm}$
$R_{n1} = 5 \text{ M}\Omega$	$\tau_A = 7.23 \times 10^{-5} \text{ sec}$
$R_{n2} = 5 \text{ M}\Omega$	$\tau_B = 5.30 \times 10^{-7} \text{ sec}$
$R_{na} = 40 \text{ M}\Omega$	$\tau_C = 2.27 \times 10^{-7} \text{ sec}$
$C_{n1} = 1.5 \text{ pf}$	$\tau_D = 7.23 \times 10^{-8} \text{ sec}$
$C_{n2} = 1.5 \text{ pf}$	$\tau_E = 7.23 \times 10^{-8} \text{ sec}$
$C_{na} = 1.5 \text{ pf}$	$\tau_{CA} = 4.15 \times 10^{-6} \text{ sec}$
$R_L = 10 \text{ M}\Omega$	$\tau_{CB} = 8.11 \times 10^{-7} \text{ sec}$
$R_{c1} = 100 \text{ K}\Omega$	$G_1 = \text{Variable } 0 - 1000$
$R_{c2} = 100 \text{ K}\Omega$	$G_2 = \text{Variable } 0 - 100$
$R_{c3} = 100 \text{ K}\Omega$	$x = 0.2 \text{ cm}$
$R_m = 0.16 \text{ M}\Omega\text{-cm}^2$	$d = .001 \text{ cm}$
$C_m = .004 \text{ }\mu\text{f/cm}^2$	

Figure B.6 shows the magnitude portion of the Bode Plot calculated for the transfer function of equation 3.70. This plot shows the frequency response for the case where the potential control amplifier gain was 1000 and the clamping amplifier gain was 100. Several other plots were made for various values or combinations of gain and no

instabilities were noted.

The last portion of the calculations concerned the step response of the total clamping system. For this plot, the output to be observed was the current passing through the membrane at the active node, N_2 , in response to a step input clamping voltage. Since the transmembrane current, I_m , is equal to $(I_2 - I_1)$, the transfer function used previously had to be altered slightly. Solving the loop equations 3.65 to 3.68 for $(I_2 - I_1)/V_c (= I_m/V_c)$ the following result was obtained:

$$\frac{I_m}{V_c} = \frac{K_c (KR_L - z_2)}{(z_1 + z_3) (z_2 - KR_L + z_3) + z_3 [KR_L (K_c + 1) - z_3]} \quad (3.72)$$

The same procedure for obtaining the step response used for the potential control system was applied here. The input was the same (see Figure B.5) and again was represented by its Fourier series to 60 harmonics. The current flowing through the membrane for a 1 volt input clamping voltage is shown in Figure B.7. Again the initial overshoot and oscillations are the result of the Gibbs effect previously discussed. Figure B.7 indicates that the system may possibly have a sustained oscillation; however, since the oscillations are the same frequency as the Gibbs phenomena, it is probably an extension of this. The step response was plotted for different amplifier gains and, although the values of current changed, the general shape of the waveforms remained

much the same.

Attempts were made in the stability analysis of both the potential control system and the clamping system to observe the effects of changing the length of the internode in the grounded pool. The assumptions necessary to treat the system in the above manner made it quite difficult to investigate this factor meaningfully. In any case the length was reduced from 0.2 cm to 0.02 cm and Bode Plots for both systems altered only very slightly.

CHAPTER IV

EXPERIMENTAL RESULTS

Single nerve fibres were dissected by Dr. R. S. Smith of the Surgical Medical Research Institute of the University of Alberta from the sciatic nerve found in the leg of the frog, *Xenopus Laevis*. The single fibres were then mounted in the nerve mounting chamber and petroleum jelly seals were placed as indicated in the photo of Figure 4.1(a). Connections were then made to the amplifier inputs by means of salt bridges going to calomel cells. The photo of Figure 4.1(b) shows the entire clamping system ready for operation.

Due to the small size of the nerve fibre (10-20 microns in diameter) it was extremely difficult to manipulate the fibre and still keep it in good condition. Consequently experimentation of this sort required many attempts before suitable results could be obtained.

A. Potential Control System

Several experiments were conducted to observe the performance of the potential control system. For this purpose, the clamping amplifier was disconnected altogether, the current measuring amplifier was connected such that it measured the potential across seal B-C. The potential across seal B-C was actually the error signal to the feedback amplifier. The pulse generator was operated in the

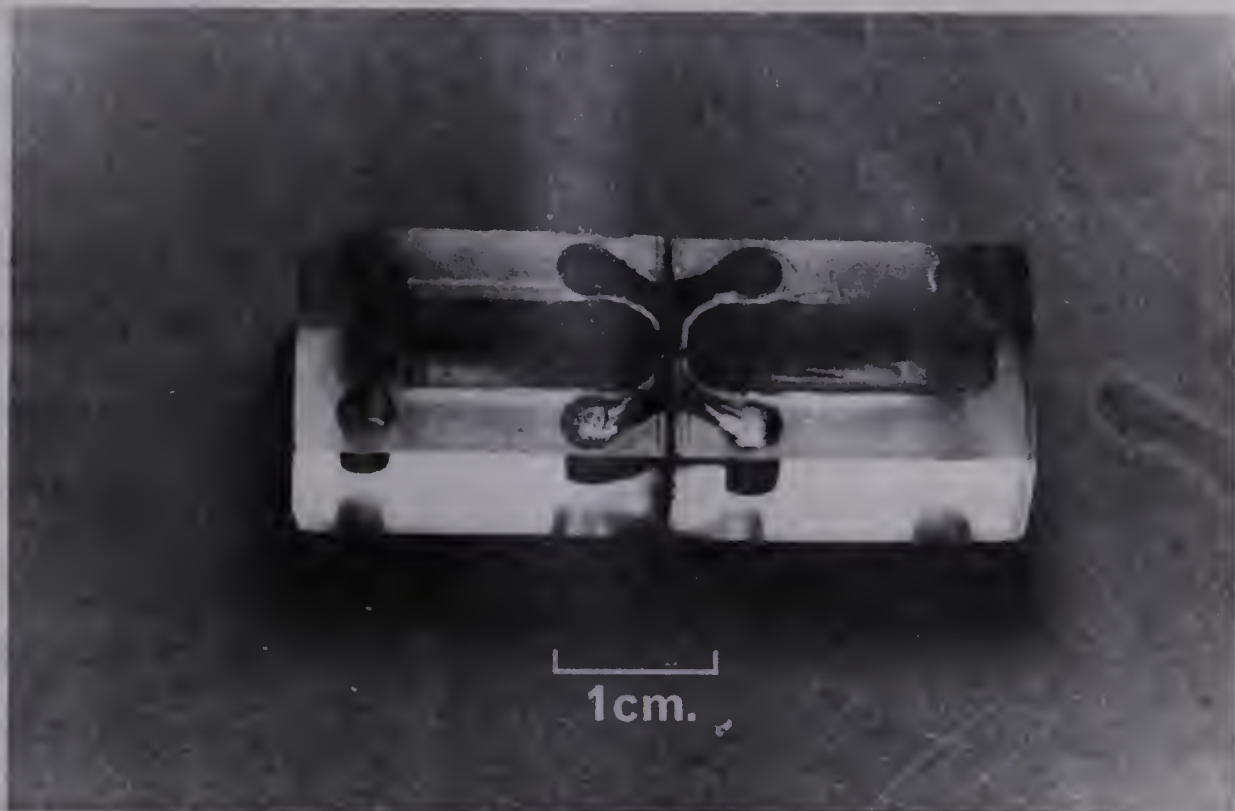


FIGURE: 4.1 (a) NERVE MOUNTING CHAMBER

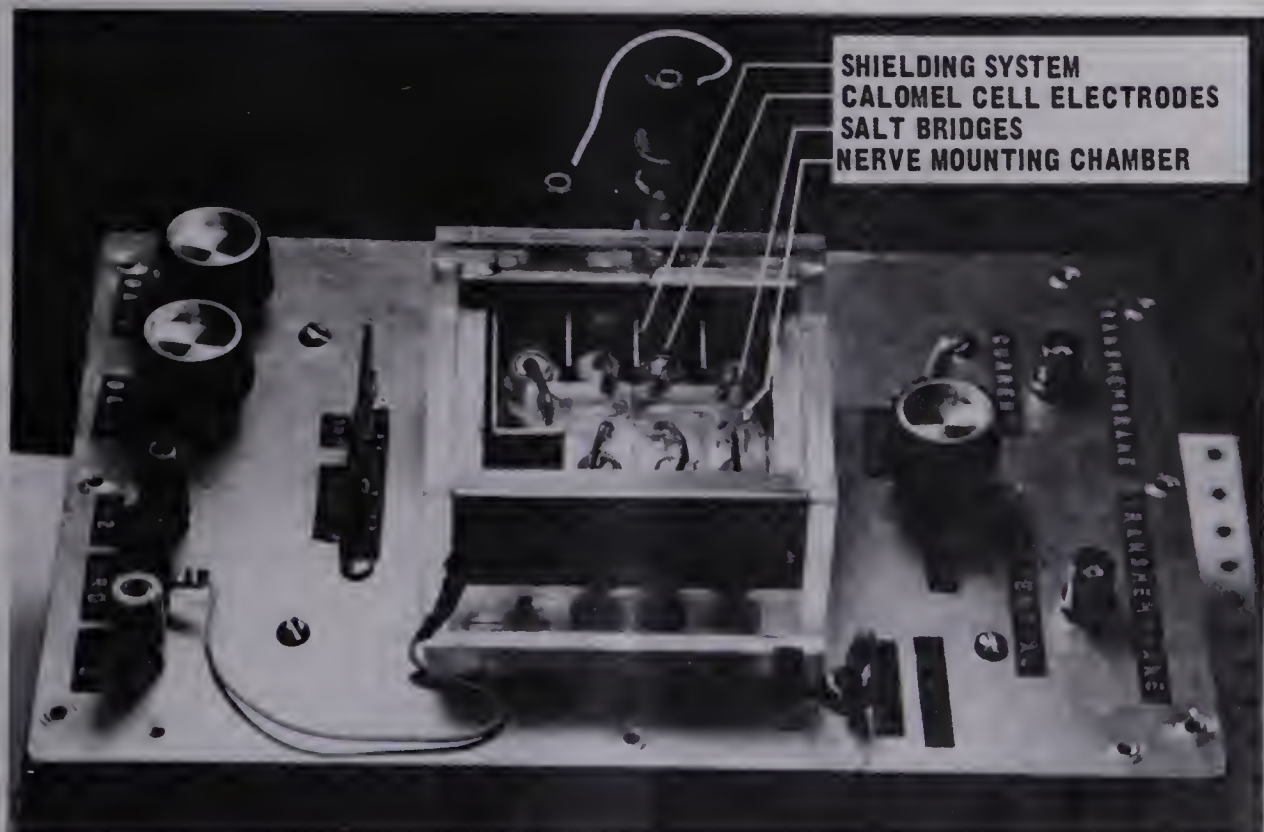


FIGURE: 4.1 (b) CLAMPING SYSTEM

manual mode and the pulse width was set in the 'stimulate' range. The amplitude was then adjusted such that an action potential was initiated in the node, N_2 . Figure 4.2(a) shows the potentials measured across the seals when the feedback amplifier gain was set to a low value. The recorded potentials indicate, although not too clearly, the longitudinal current flowing along the exterior of the fibre. As the gain was turned up the longitudinal current decreased due to negative feedback in pool A. At a certain point in the gradual increase of gain, the feedback was enough to stop sufficient current such that an action potential could be recorded across Seal A-B as shown in Figure 4.2(b). As the gain was turned up even further, the error signal decreased still more and the recorded action potential increased slightly. When the gain reached approximately 1000, the system was observed on occasions to go unstable and oscillate at a frequency of about 100-150 KHz.

Several problems were encountered in recording action potentials in this manner. The first few experiments failed due to the fact that the nerve fibre was either dead or depolarized before any experimentation could be undertaken. Depolarization of the active node could occur due to potassium ions drifting from the saturated KCl solution located inside the capillary tubes, used for salt bridges, into the pools. Care was taken in the construction of the salt bridges to melt the ends down until only

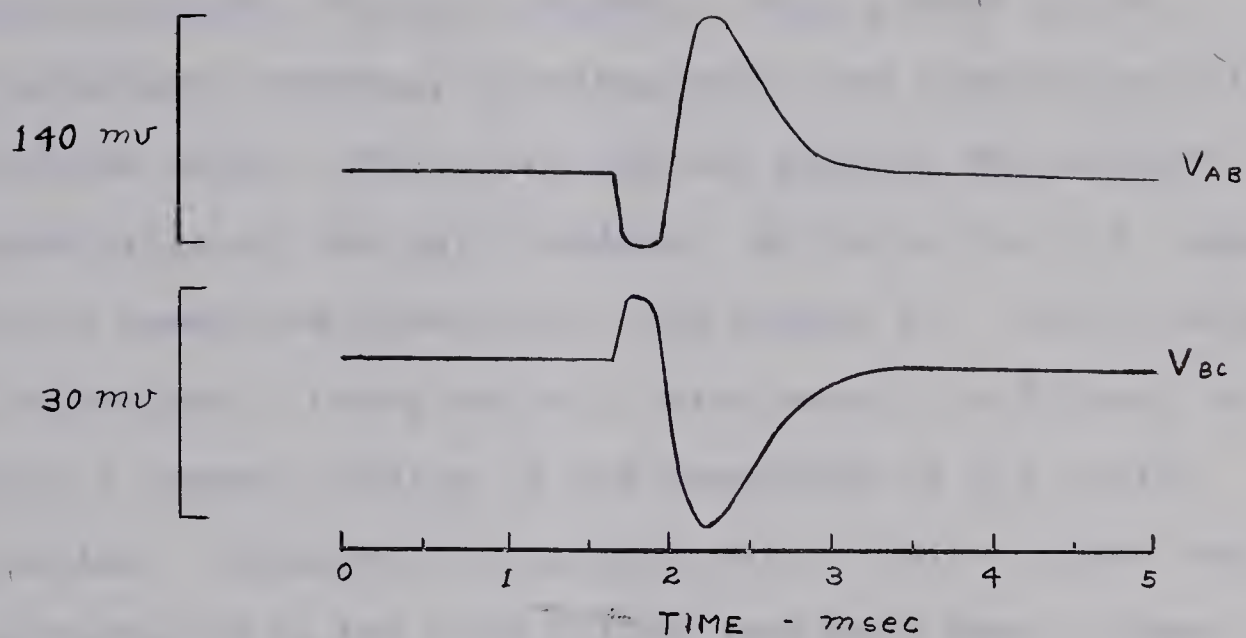


Figure 4.2(a)

Potentials Across Seals
Resulting From a Low Feedback Amplifier Gain

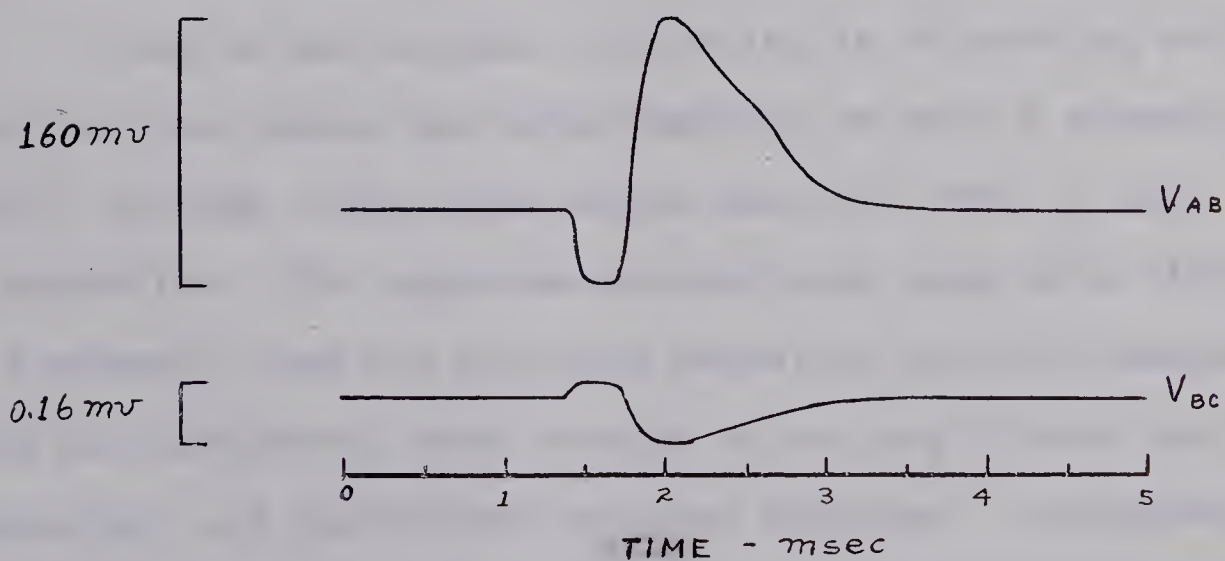


Figure 4.2(b)

Potentials Across Seals
Resulting From a Feedback Amplifier Gain of 1000

a fine hole remained. It appears though that the initial attempt was not sufficient. Chlorided silver wires were used to replace the salt bridges. This proved quite satisfactory; however, problems were then encountered with electrode drift. The wires did not possess the current capabilities of the salt bridges. By using the D.C. bias control described previously, the proper D.C. level could be maintained. Using the D.C. bias made it difficult to obtain a proper reading of the magnitude of the action potential. Consequently another set of salt bridges was constructed with the ends melted down even more. These proved to be more satisfactory in operation.

B. Voltage Clamping System

Due to the extreme difficulty in dissecting and mounting the fibres and experimenting on such a microscopic scale, voltage clamp experiments were not done to any satisfaction. The experiments were even much more difficult to implement than the previous potential control experiments. From the laboratory test results on the amplifiers and pulse generator, all specifications were achieved. Consequently it is felt that little problem with the amplifier system would have been encountered. Other problems due to the effects of capacitive coupling, electrode polarization, etc., could not be evaluated since the experiments were not attempted to any satisfactory degree.

The calculations on the voltage clamping system indicate that the voltage clamp apparatus would have operated satisfactorily. No instabilities appeared in the calculations other than those of the potential control system. By maintaining the potential control amplifier away from the unstable point, no problem was foreseen.

CHAPTER V

CONCLUSIONS

The first portion of this project, i.e. building the voltage clamp system, was achieved. The design and construction of all the electronic apparatus necessary to carry out experiments was completed. Laboratory testing indicated that all the specifications initially laid out were achieved and in some cases, exceeded. From these tests and from actually using the apparatus in the measurement of action potentials, it can be concluded that the system, as constructed, is capable of conducting voltage clamp experiments to the required accuracy.

The calculations carried out on the system stability, which was the prime concern of the analytical study, indicated that the potential control system could go unstable at a gain of approximately 300 at which it oscillated at a frequency of approximately 360 KHz. This system was observed experimentally to go unstable at a gain of about 1000 where it oscillated at a frequency of approximately 100 KHz. Although the two observations do not seem to agree very well, it must be kept in mind that it is rather difficult to correlate the actual observations with the computed results. Values of the parameters used to represent the entire system electrically were selected from various papers which estimated their values. Actually measuring them was beyond the purpose of this study. It

can be appreciated, also, that the parameters varied from fibre to fibre as was evidenced by the observation of the fibre diameters varying from fibre to fibre. The resistances due to the petroleum jelly seals could obviously not be set to the same values each time.

Calculations involving the total clamping system indicate that no points of instability occur other than the point of instability located in the potential control system.

From the calculations involving the potential control system, one of the most important conclusions that can be stated is that the stability for this system is dependent on gain. This is illustrated by Figure B.2 of Appendix B.

The effect of the length of internode in the grounded pool was mathematically investigated. The results showed that changing this length had little effect on the stability of the system.

It is felt that more accurate analytical results could have been obtained if the system was approached mathematically in the first manner described, i.e., to compute in the time domain solving numerically the numerous simultaneous differential equations necessary to describe the system. This approach would not have necessitated the adoption of the several assumptions outlined. A solution of this type, however, would have been extremely complex and beyond the scope of this thesis project.

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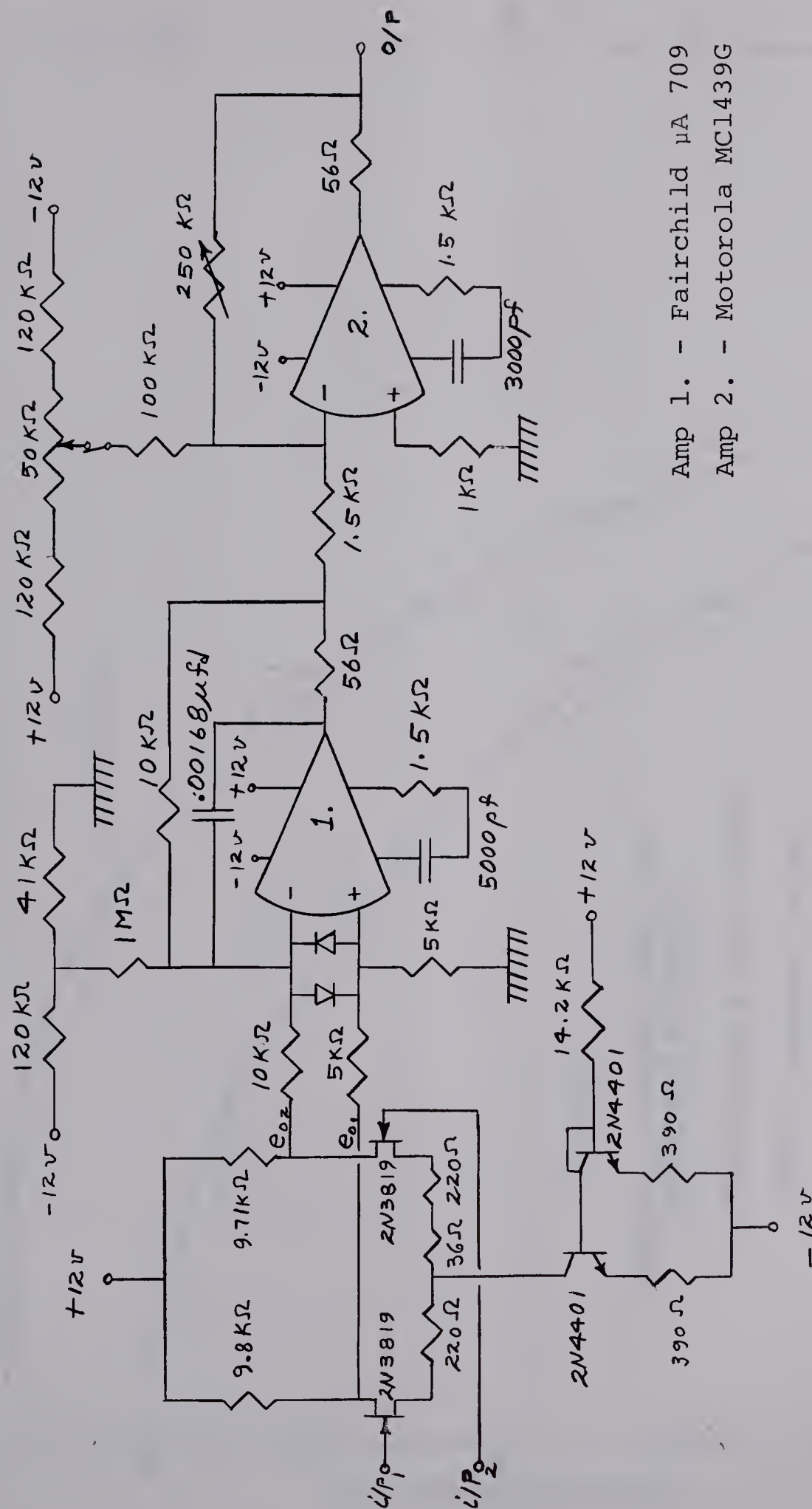
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APPENDIX A

CIRCUIT DIAGRAMS FOR CLAMPING SYSTEM
AND FOR VOLTAGE GENERATOR SYSTEM

FREQUENCY RESPONSE FOR POTENTIAL CONTROL AMPLIFIER
AND FOR CLAMPING AMPLIFIER



Amp 1. - Fairchild μA 709
Amp 2. - Motorola MCL439G

Figure A.1
Potential Control Amplifier

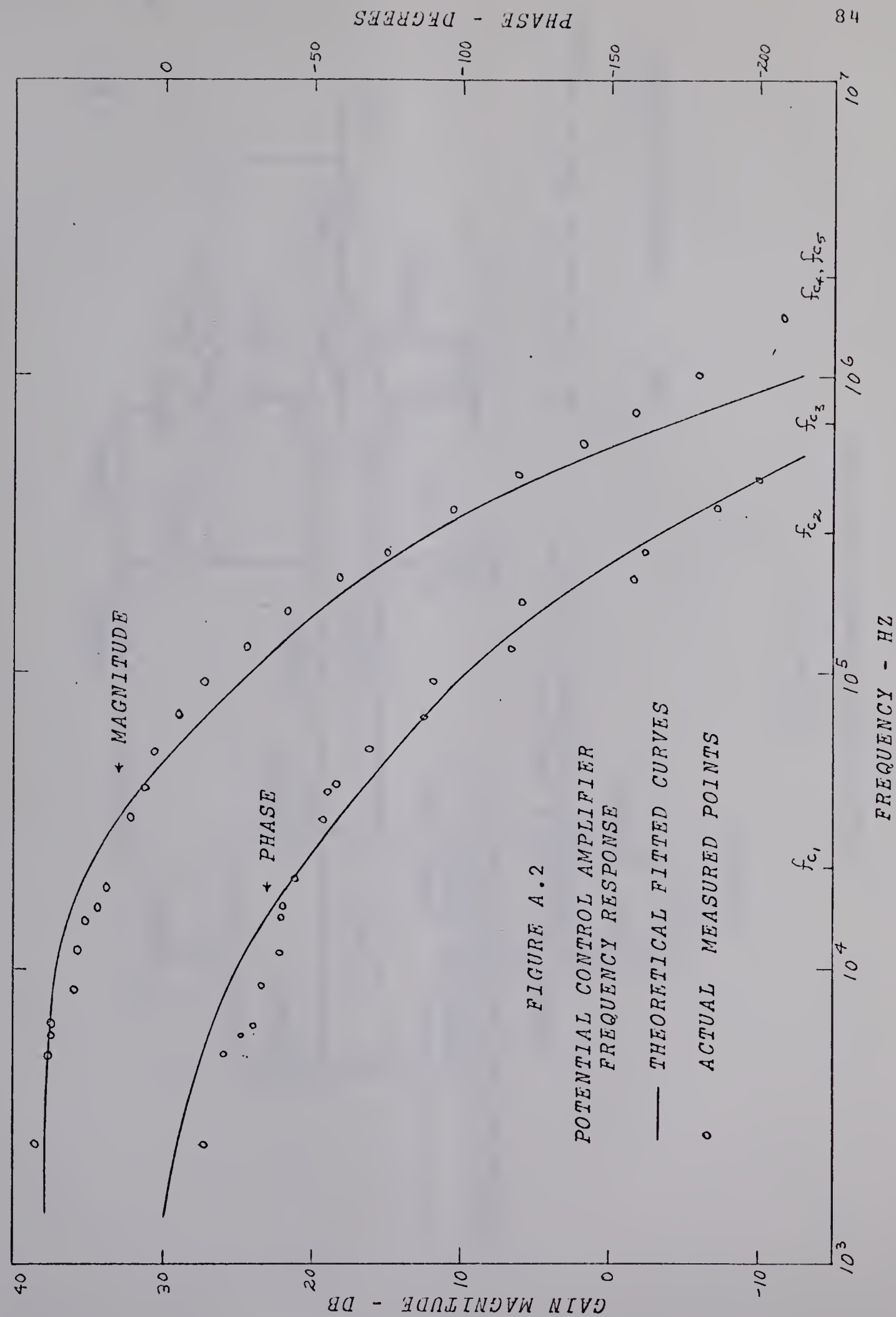


FIGURE A.2

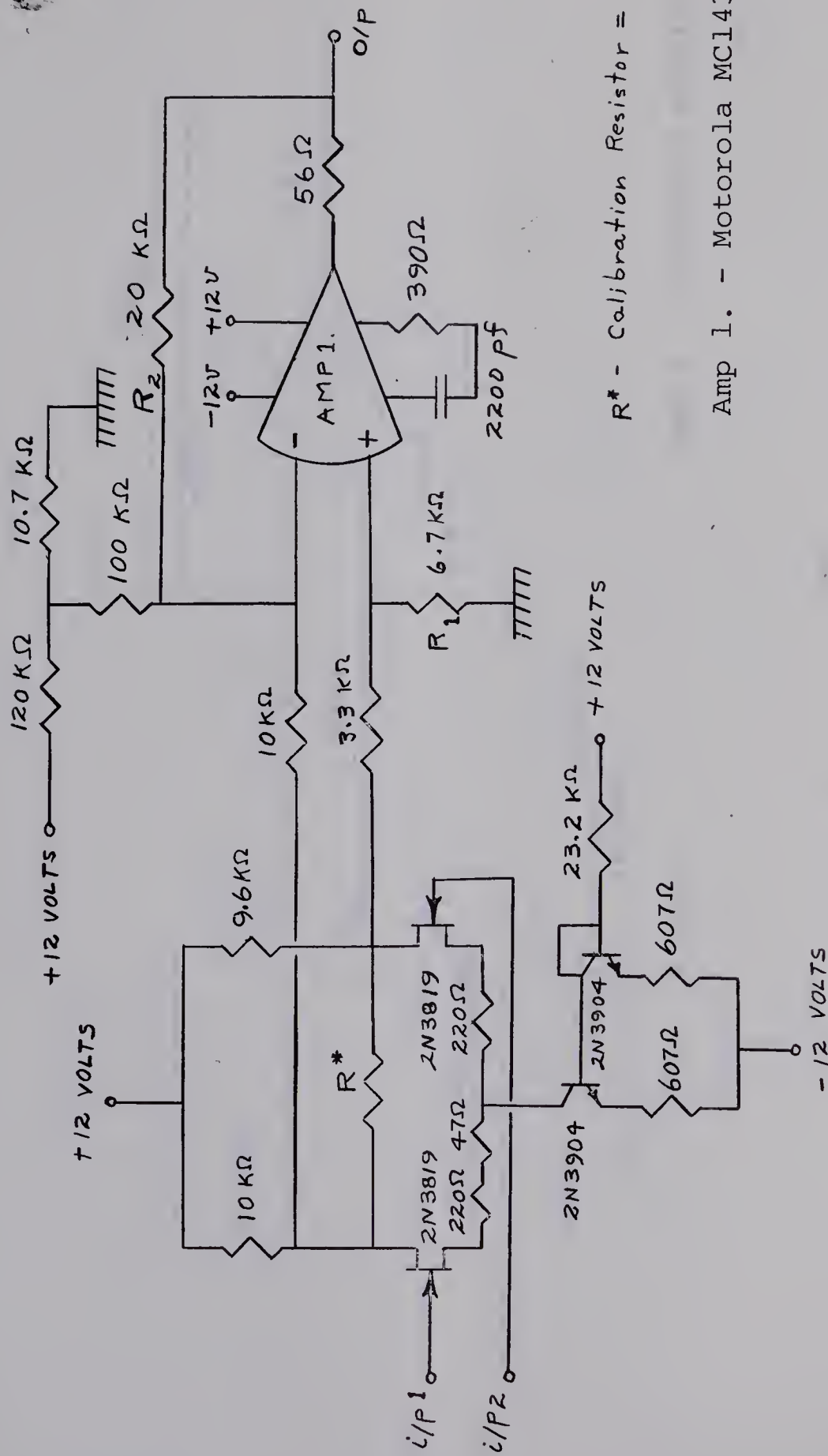
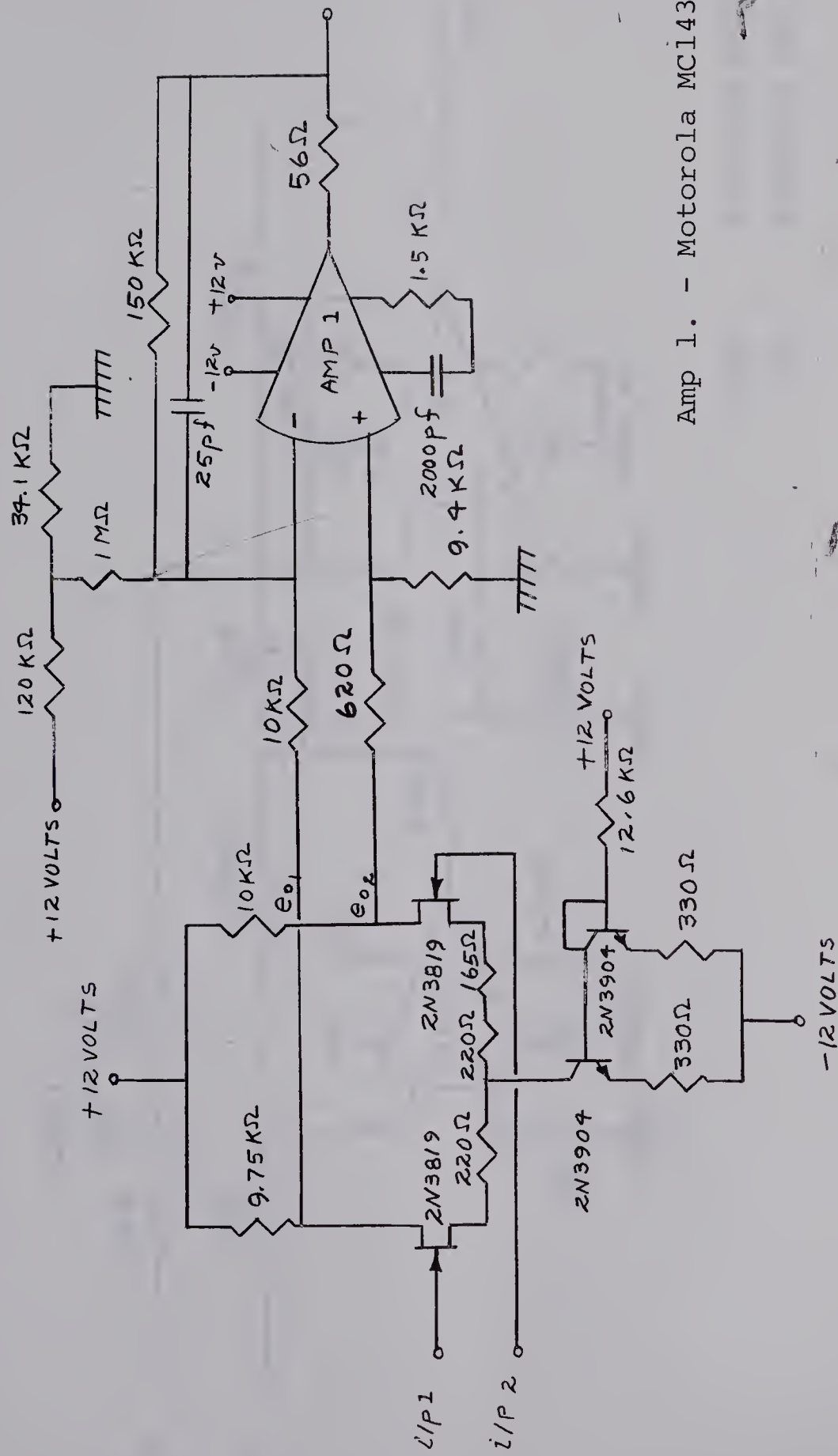
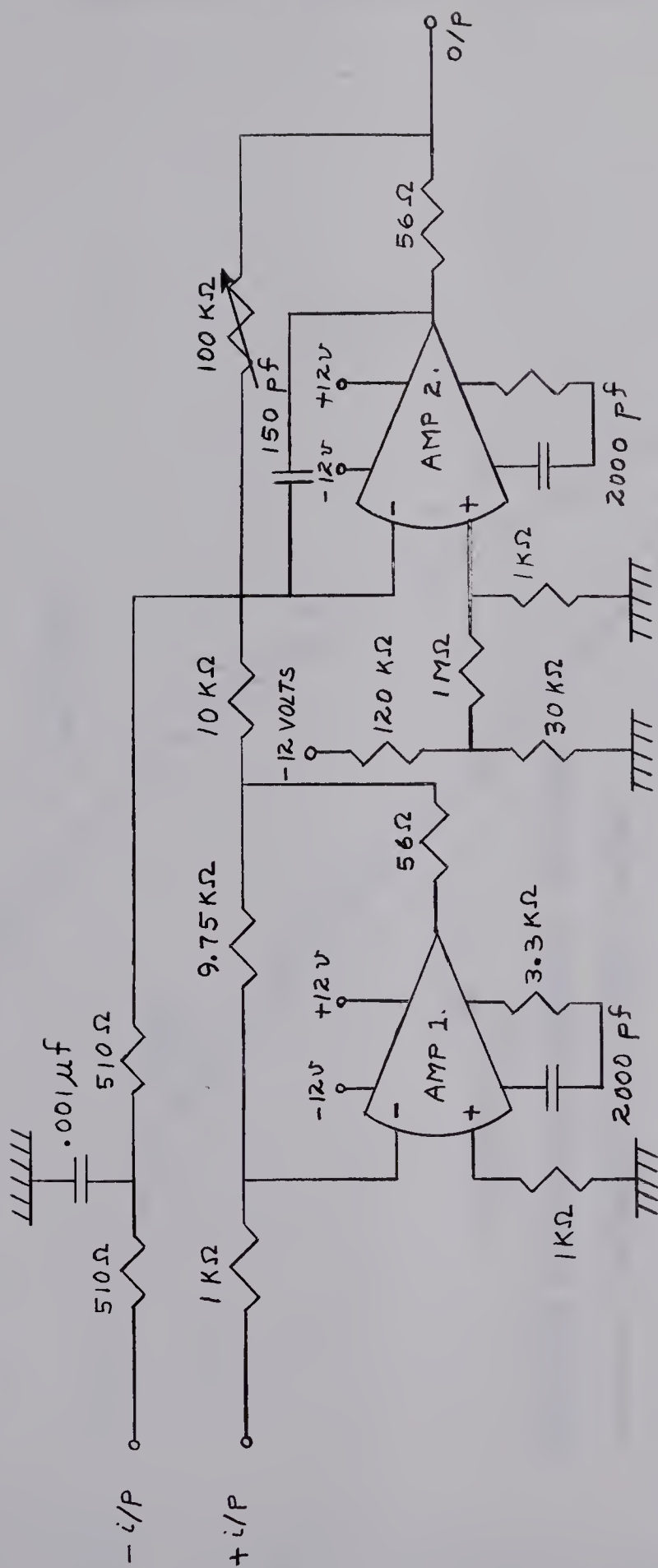


Figure A.3
Potential Measurement Amplifier



Amp 1. - Motorola MCL439G

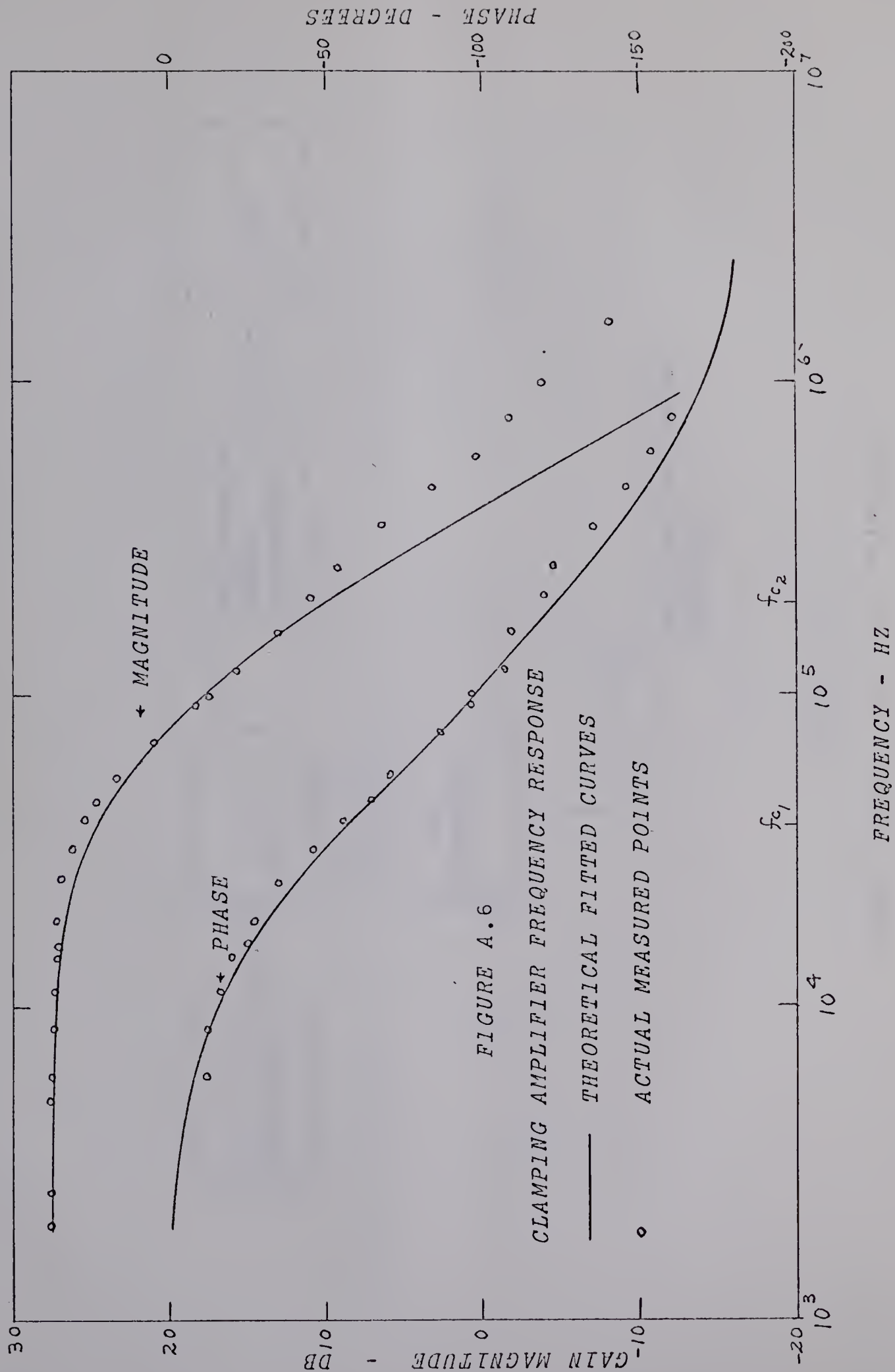
Figure A.4
Current Measurement Amplifier



Amp 1. - Motorola MC1439G

Amp 2. - Motorola MC1439G

Figure A.5
Clamping Amplifier



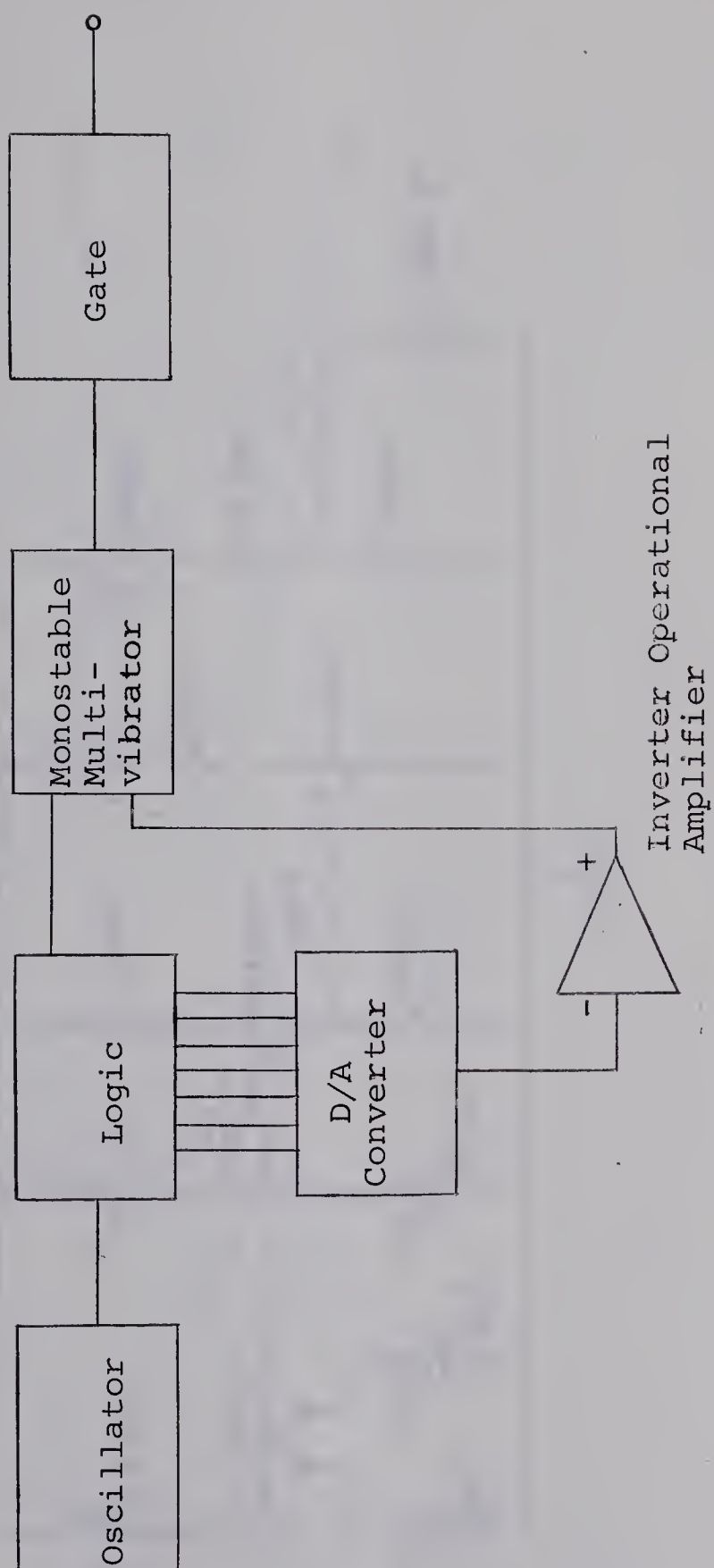


Figure A.7

Block Diagram for Nerve Stimulator

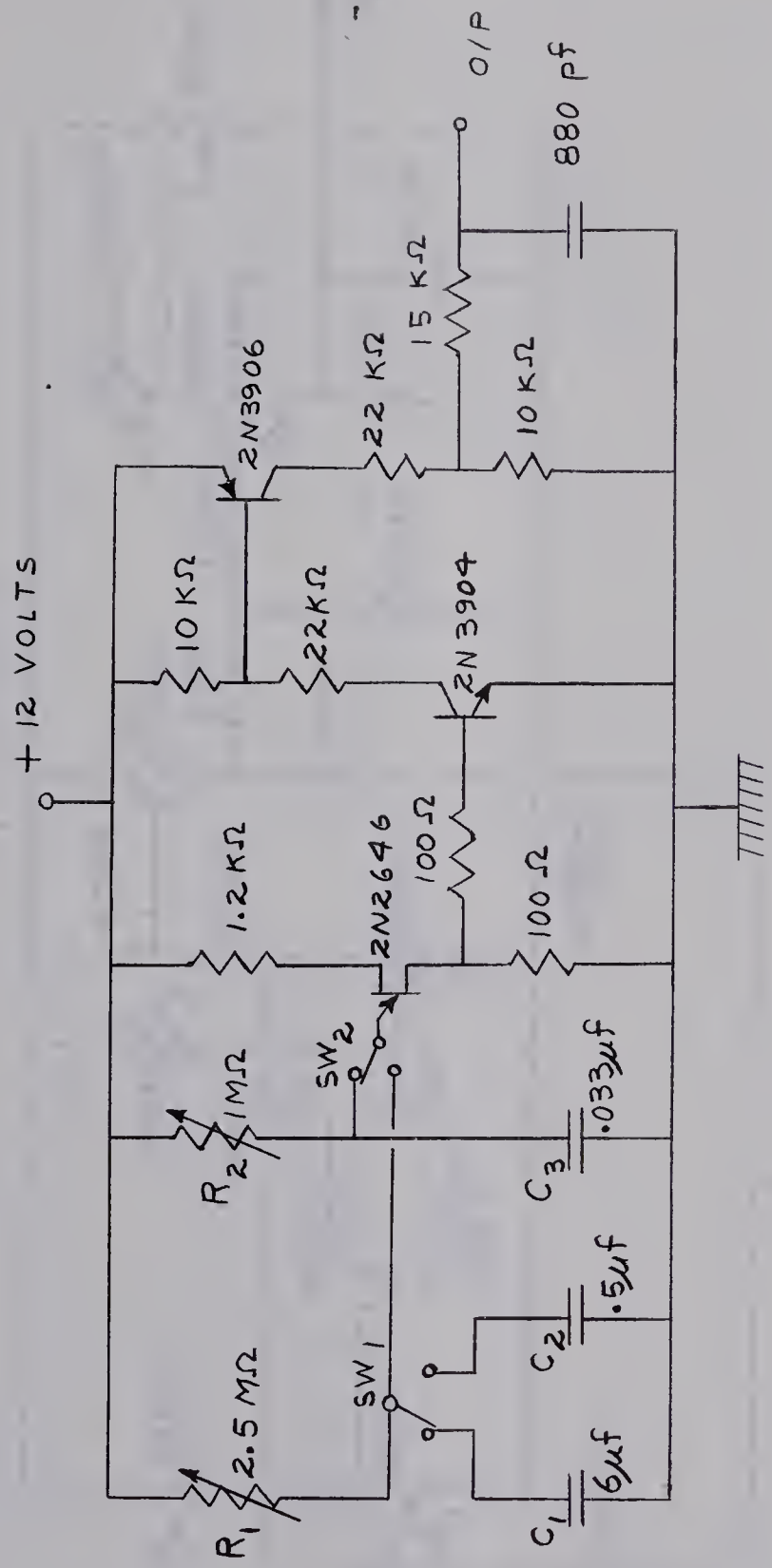


Figure A.8
Trigger Circuit

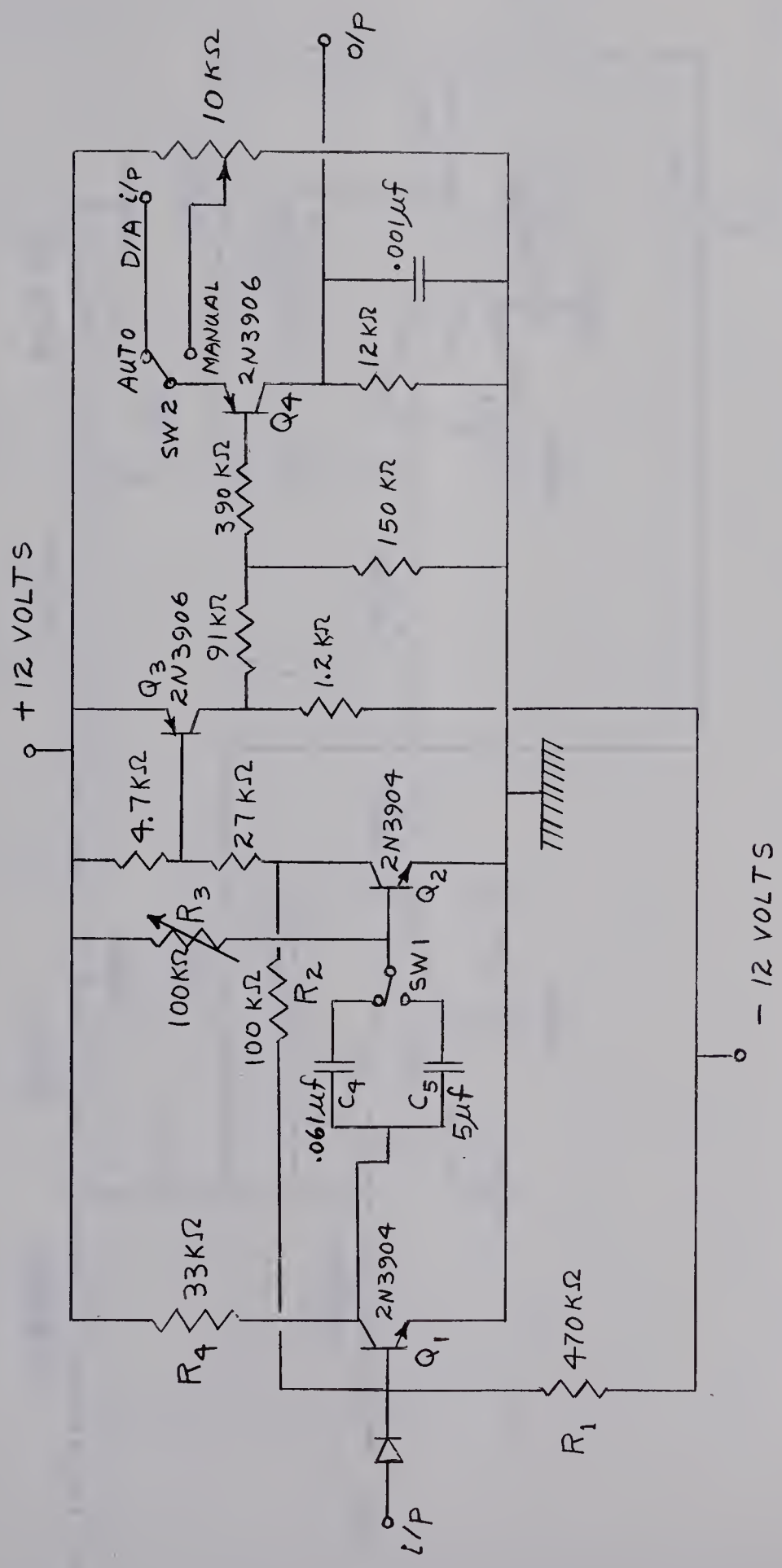


Figure A.9
Monostable Multivibrator Circuit

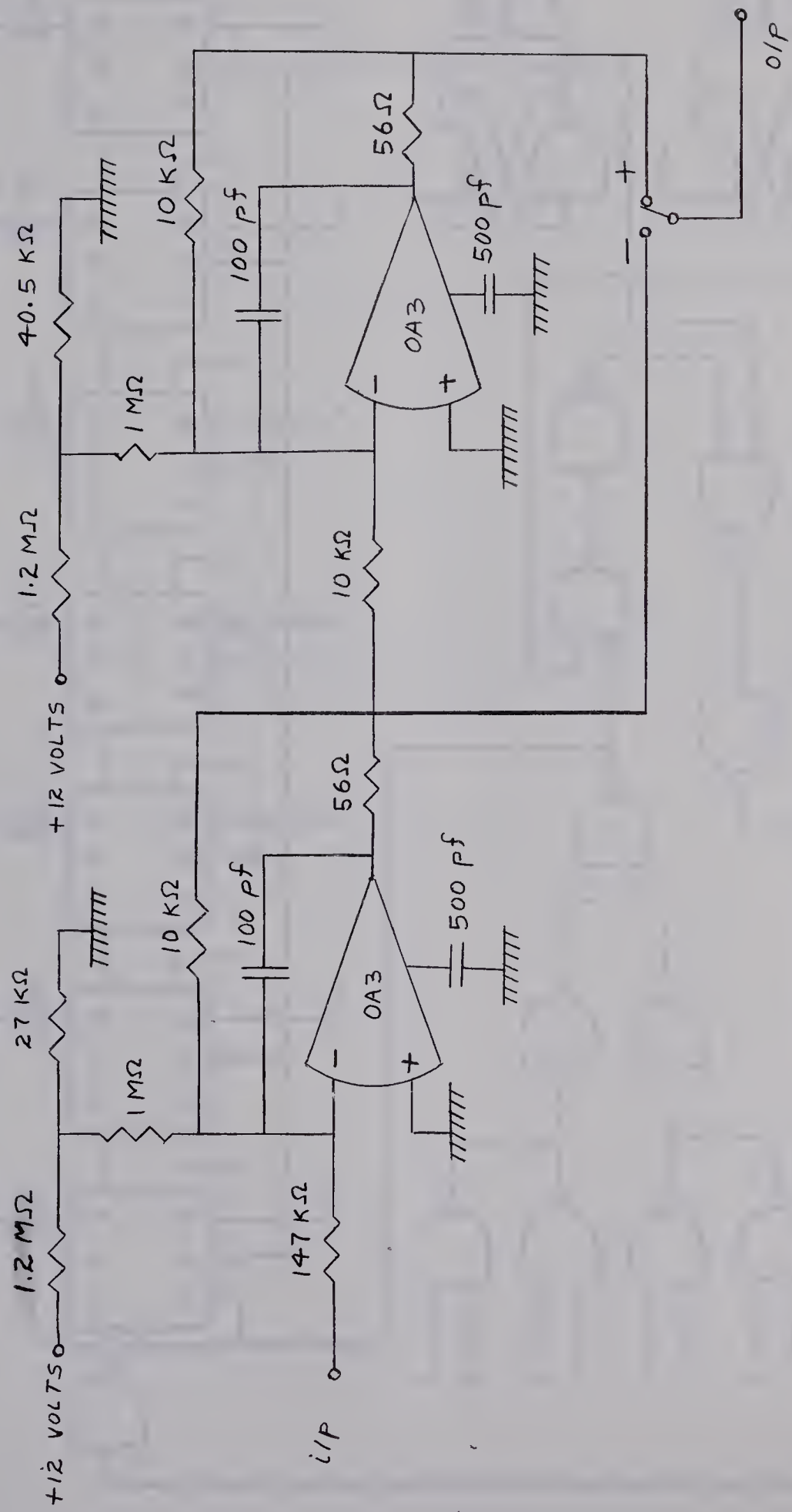


Figure A.10

Gate Circuit

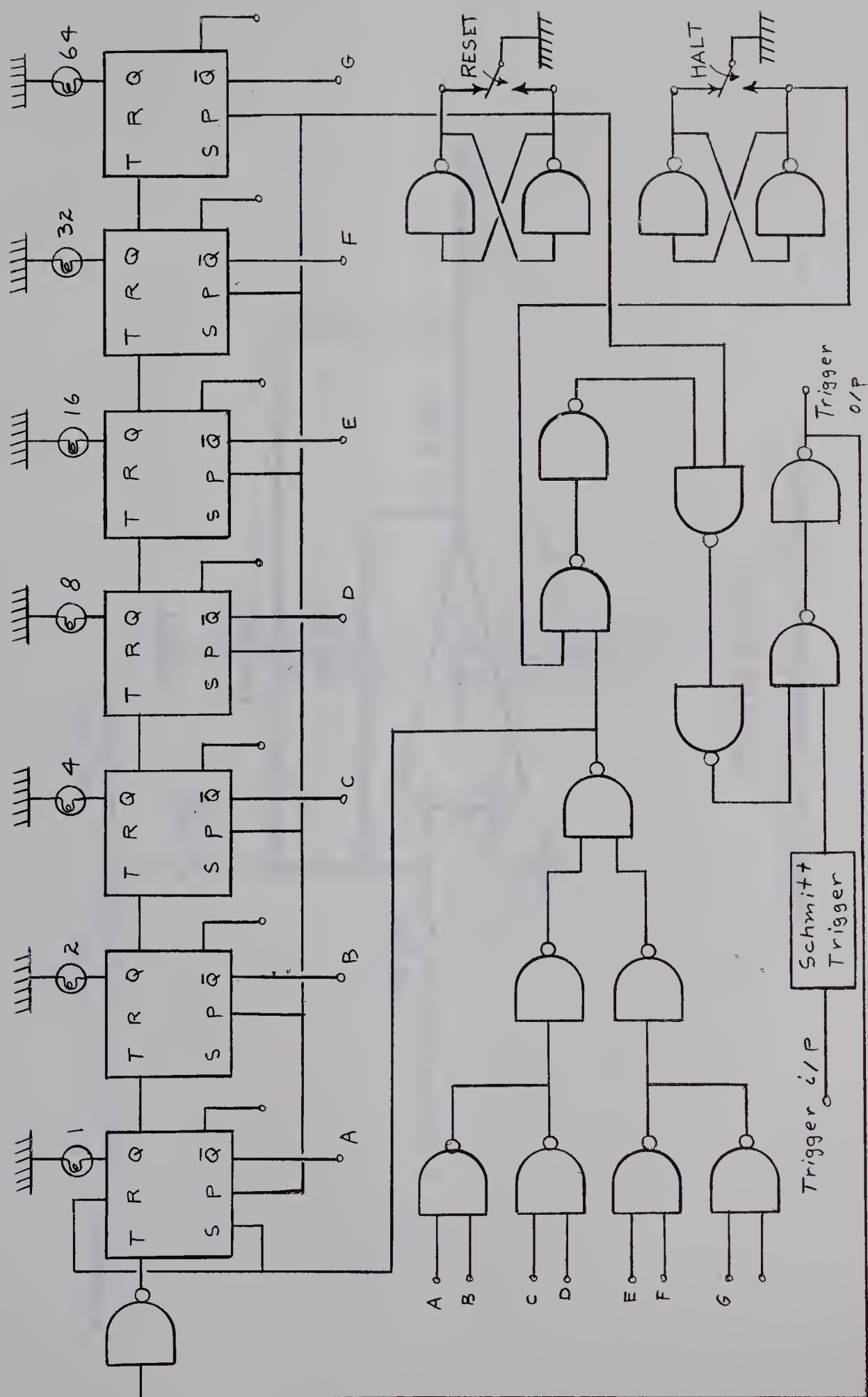


Figure A.11

Logic Connections

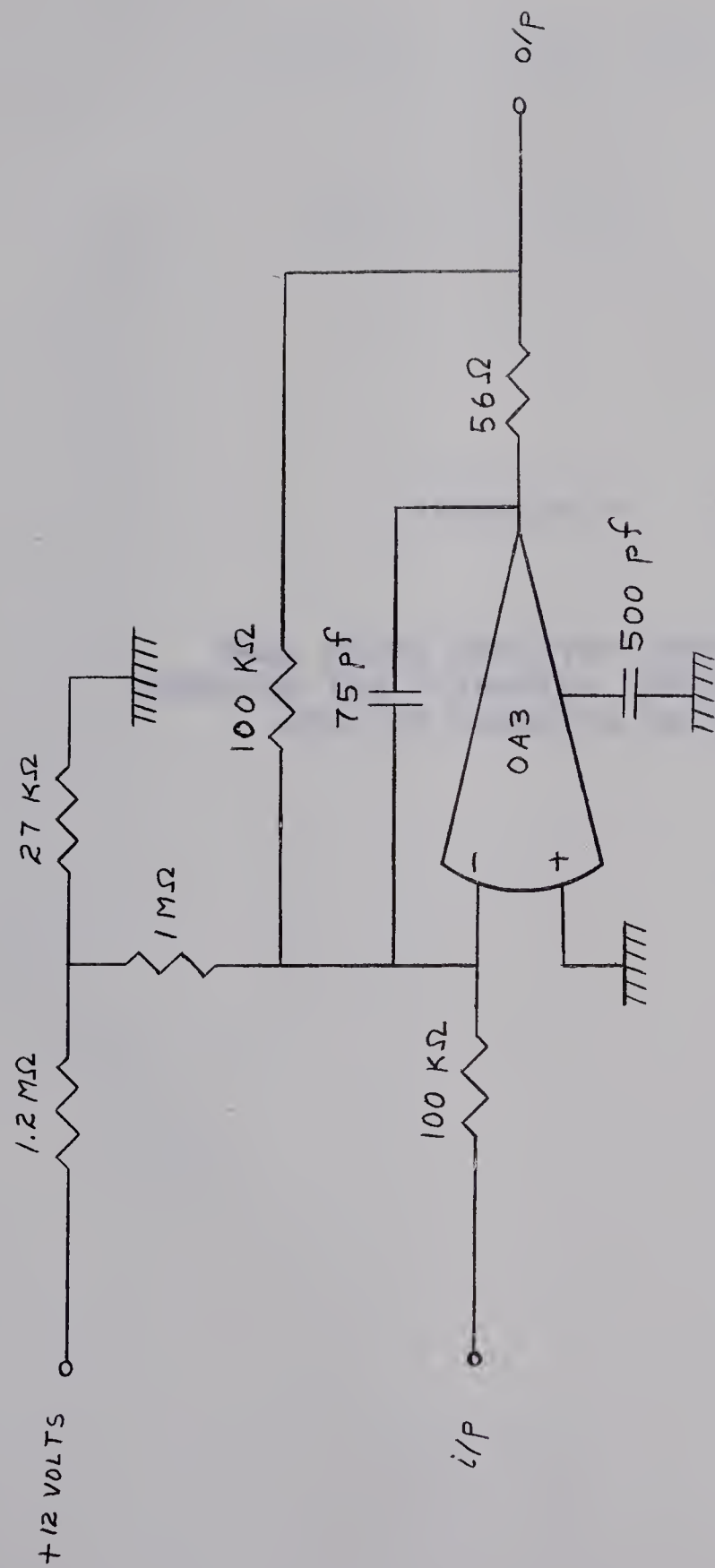


Figure A.12
Inverting Operational Amplifier

APPENDIX B

BODE PLOTS AND STEP RESPONSES
COMPUTED FOR POTENTIAL CONTROL SYSTEM
AND FOR CLAMPING SYSTEM

MAGNITUDE OF GAIN VERSUS FREQUENCY FOR
POTENTIAL CONTROL SYSTEM

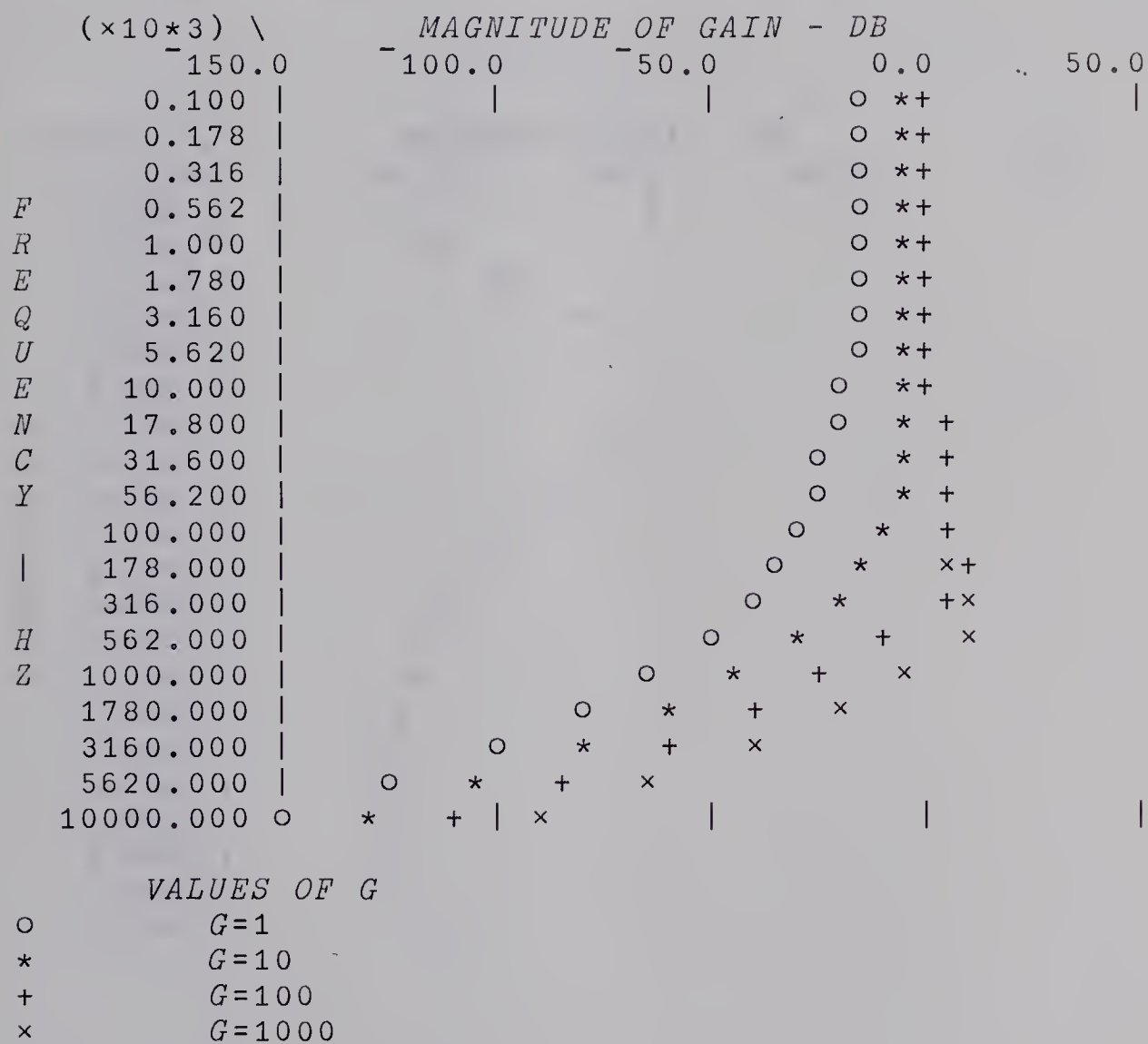


FIGURE B.1

EFFECTS OF AMPLIFIER GAIN ON THE MAXIMUM VALUE
OF VO/VA THROUGH A FREQUENCY RANGE OF 100 HZ TO 10 MHZ

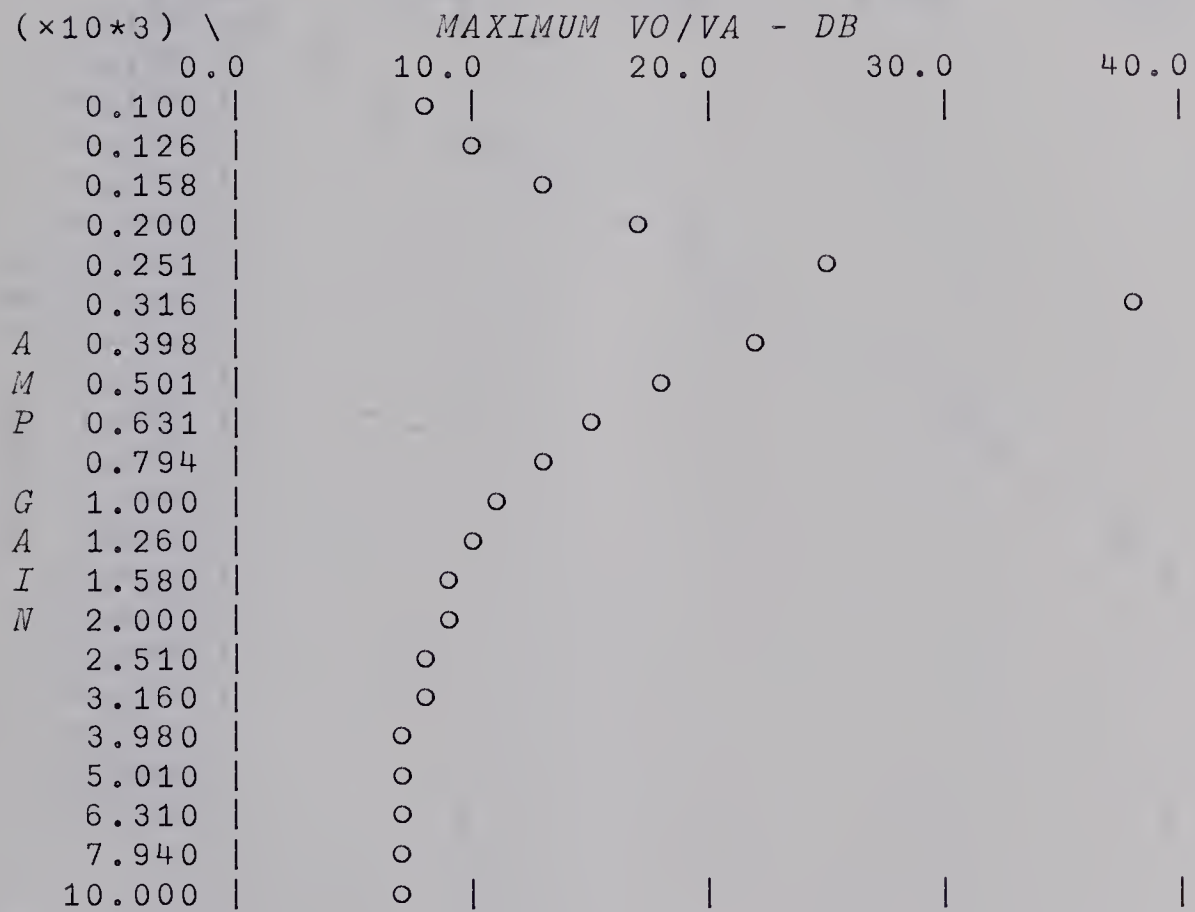


FIGURE B.2

FREQUENCY AT MAXIMUM V_O/V_A VERSUS FEEDBACK AMPLIFIER GAIN

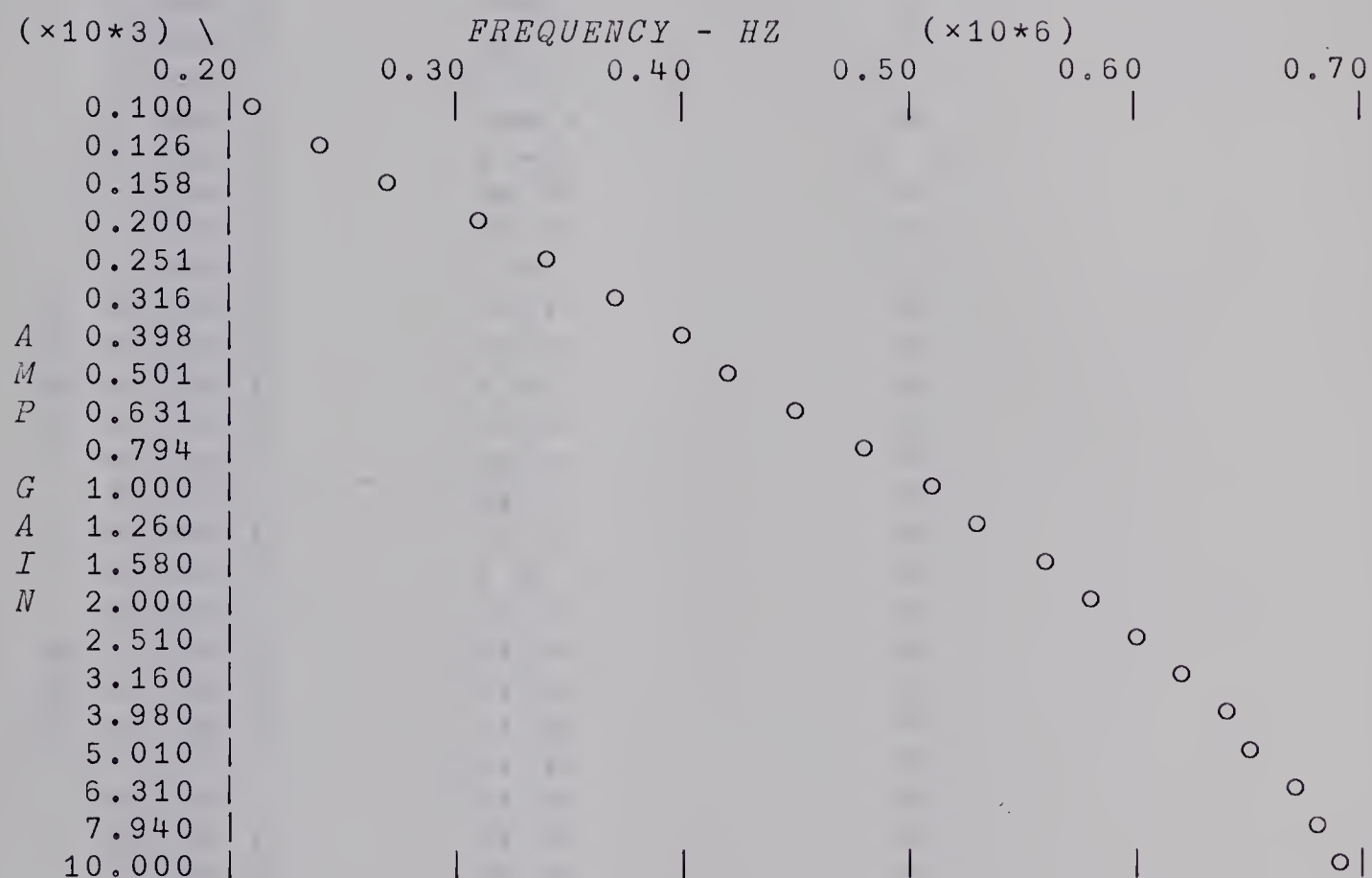
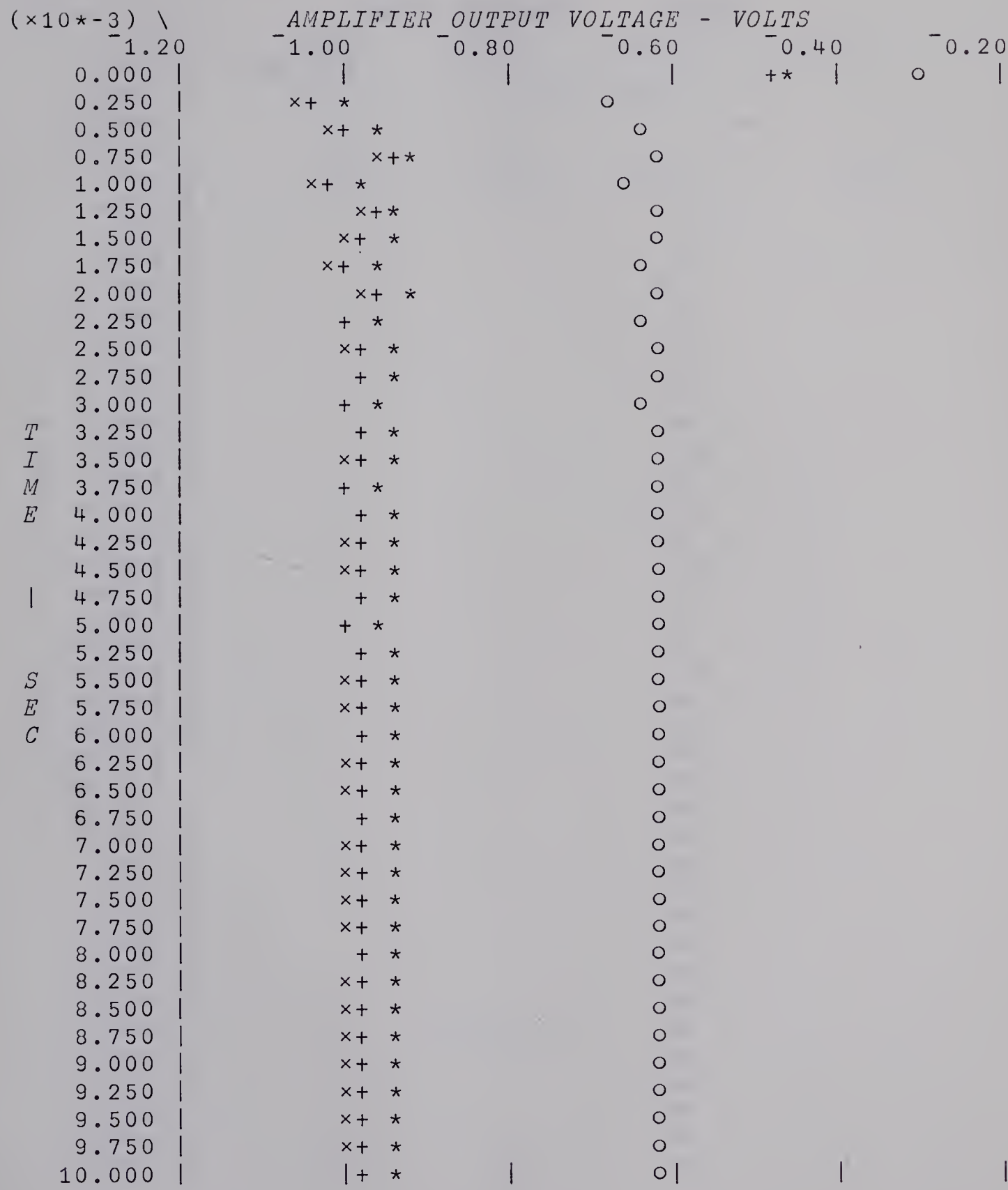


FIGURE B.3

STEP RESPONSE FOR POTENTIAL CONTROL SYSTEM



VALUES OF GAIN

○ G=10

* G=100

+ G=350

x G=1000

FIGURE B.4

STEP INPUT VOLTAGE FOR SYSTEM ANALYSIS

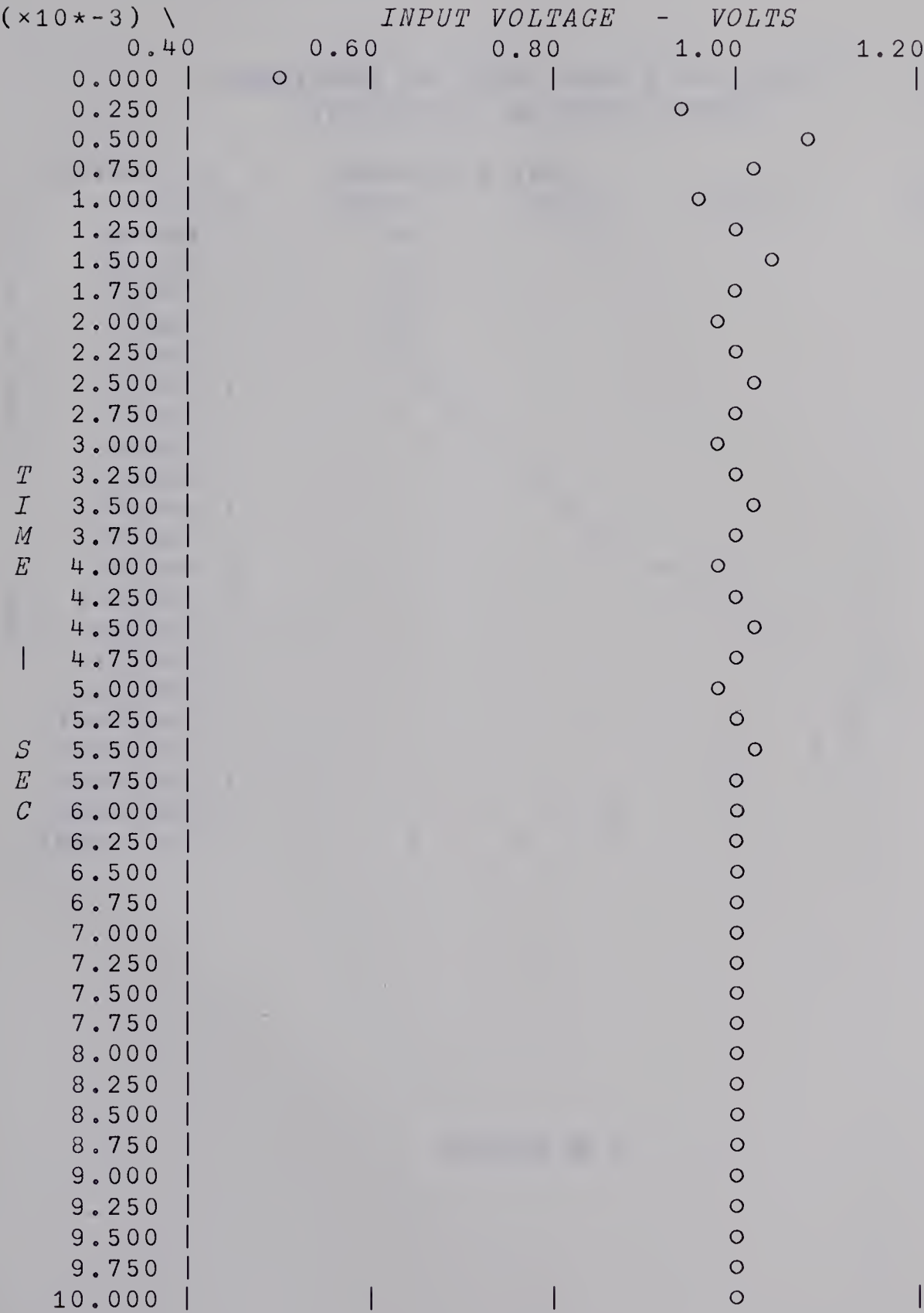


FIGURE B.5

MAGNITUDE OF GAIN VERSUS FREQUENCY
FOR TOTAL CLAMPING SYSTEM

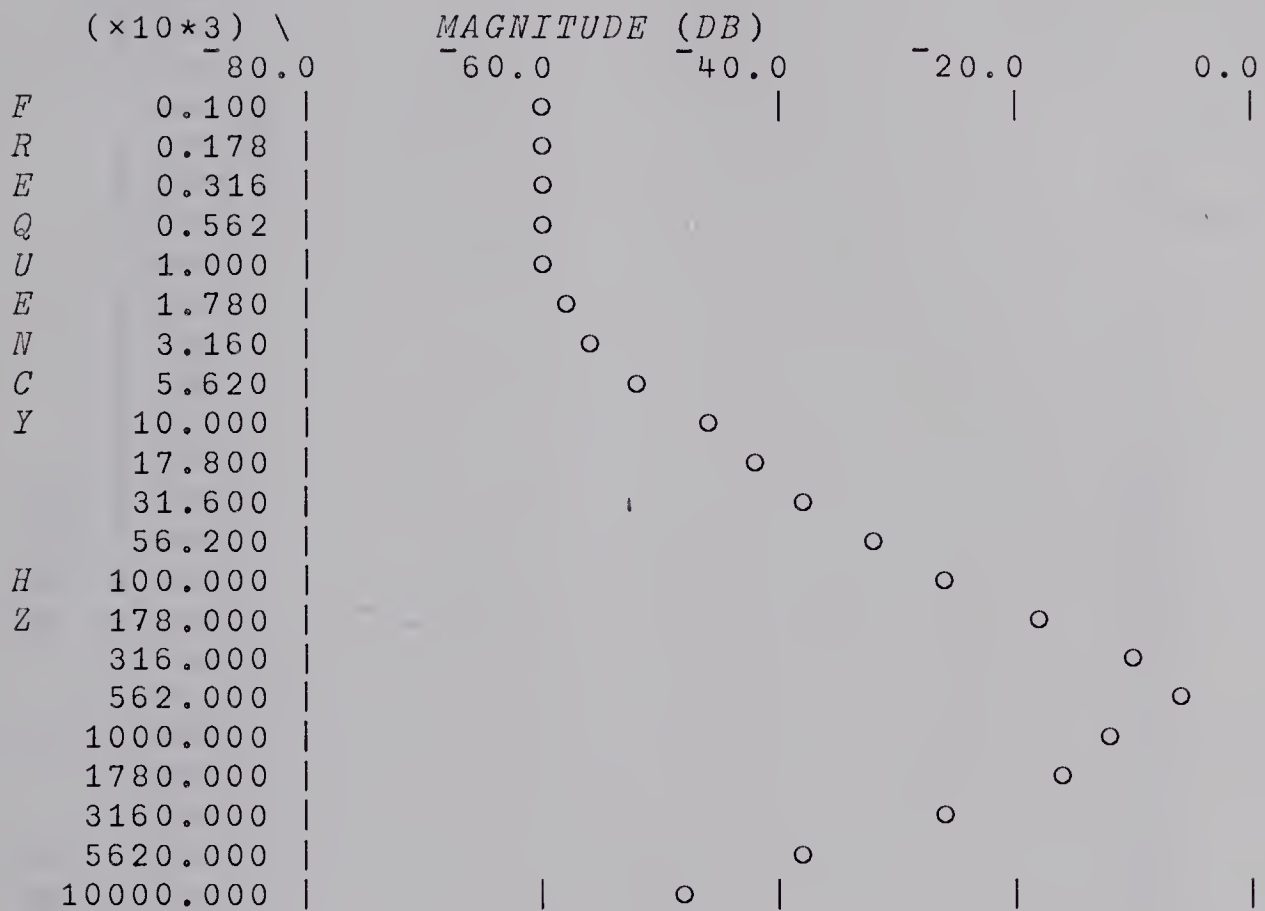
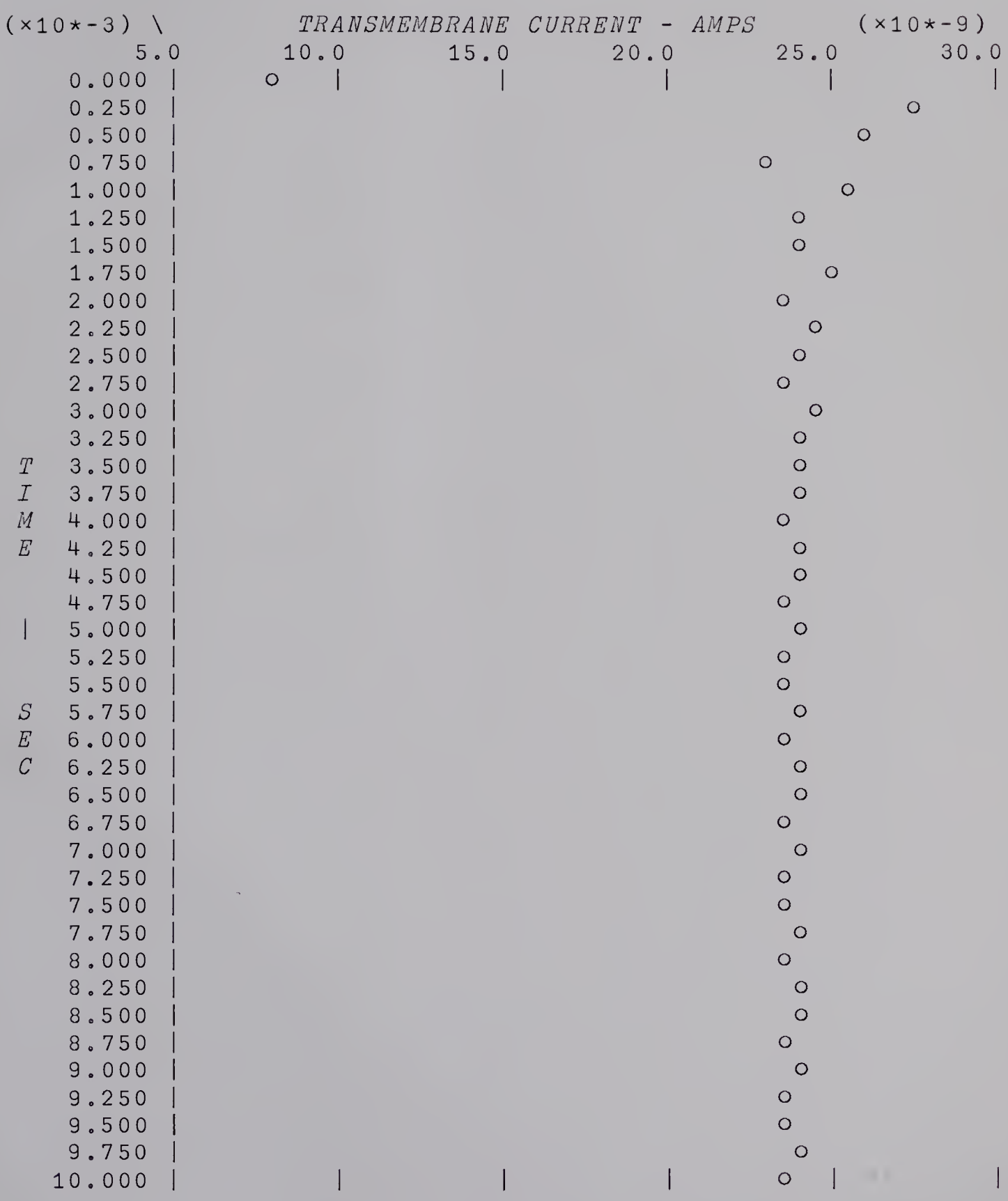


FIGURE B.6

VOLTAGE CLAMP STEP RESPONSE
TRANSMEMBRANE CURRENT FOR A UNIT STEP INPUT



○

FIGURE B.7

B29922